IOBC / WPRS

Working Group "Integrated Control in Oilseed Crops"

OILB / SROP

Groupe de Travail "Lutte Intégrée en Culture d'Oléagineux"

Proceedings of the meeting

at

Poznań (Poland)

11-12 October, 2004

Edited by

Birger Koopmann, Samantha Cook,

Neal Evans and Bernd Ulber

IOBC wprs Bulletin
Bulletin OILB srop Vol. 29 (7) 2006

The content of the contributions is in the responsibility of the authors

The IOBC/WPRS Bulletin is published by the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section (IOBC/WPRS)

Le Bulletin OILB/SROP est publié par l'Organisation Internationale de Lutte Biologique et Intégrée contre les Animaux et les Plantes Nuisibles, section Regionale Ouest Paléarctique (OILB/SROP)

Address General Secretariat:

Dr. Philippe C. Nicot
INRA – Unité de Pathologie Végétale
Domaine St Maurice - B.P. 94
F-84143 Monfavet Cedex
France

ISBN 92-9067-190-2 Web: http://www.iobc-wprs.org

Dedication

Convenors for years

At our last Working Group meeting in Poznań, October 2005, we listened to the talk given by Volker Paul about the history of our Working Group and were highly impressed by what we heard. As you may remember from the last issue of this Working Group Bulletin, Bent Bromand was the founding head of the group until 1988. At this time Volker Paul and Ingrid Williams began their convenorships. From 1988 until 2003, they organized ten (!) Working Group meetings and were editors of seven (!) IOBC-Bulletins. Both organizing meetings and editorial work are time consuming assignments. Their activities were of course an honorary post, enhancing their workload considerably. We are all obliged to Ingrid and Volker. Their activities offered the possibility to meet and have exchange with colleagues from abroad. Beside the exchange of the most recent scientific results and publishing them, the set up of a `scientific-social´ network becomes more and more important. Against this background the Working Group is of extraordinary importance to all of us. Successful funding of four EU projects (BORIS, MASTER, IMASCORE and SECURE) may also be attributed at least partly to the activities of Ingrid and Volker. These few words can only partially appreciate their achievements.

Thank you both, for all you have done for the group! Now, we hope that you are able to use your time for other nice things beside the *Working Group on Integrated Control in Oilseed Crops*. We hope that you keep in good shape, as can be seen on the photograph taken at our conference dinner in Poznań 2005.

With all our very best wishes on behalf of the whole Working Group!

Birger Koopmann and *Sam Cook*, April 2006

Preface

At our last meeting in 2004, held at Rothamsted Research, Harpenden, UK, we decided to meet next in Poznań, Poland, in 2005, to take advantage of the possibility of support provided by the EU project PAGEN. Thanks to *all* who helped the meeting to run so smoothly and in particular, Małgorzata 'Gosia' Jędryczka, for co-ordinating the local arrangements so carefully.These included an interesting and fun excursion to the Malyszyn plant breeding station of Plant Breeding Strzelce and the bat reserve Nietoperek (Miedzyrzecki Wal Umocniony, Grupa Warowna Scharnhorst).

This meeting was the biggest so far in the history of our Working Group, with 77 participants from a total of 11 countries, giving a total of 42 oral presentations and 25 posters. We are grateful to all who submitted manuscripts for the Bulletin! Editing and formatting the 48 papers and 18 abstracts in this volume was a big task, and we thank Neal Evans and Bernd Ulber for their assistance. This procedure has taken somewhat longer than we would have liked, due in part to the late submission of several papers. In order to maximize the usefulness of the Bulletin for both authors and readers, we believe we need to minimize the duration between the meeting and publication of the Bulletin. We will therefore ask that *all* manuscripts for the Bulletin are submitted *at the meeting* in future.

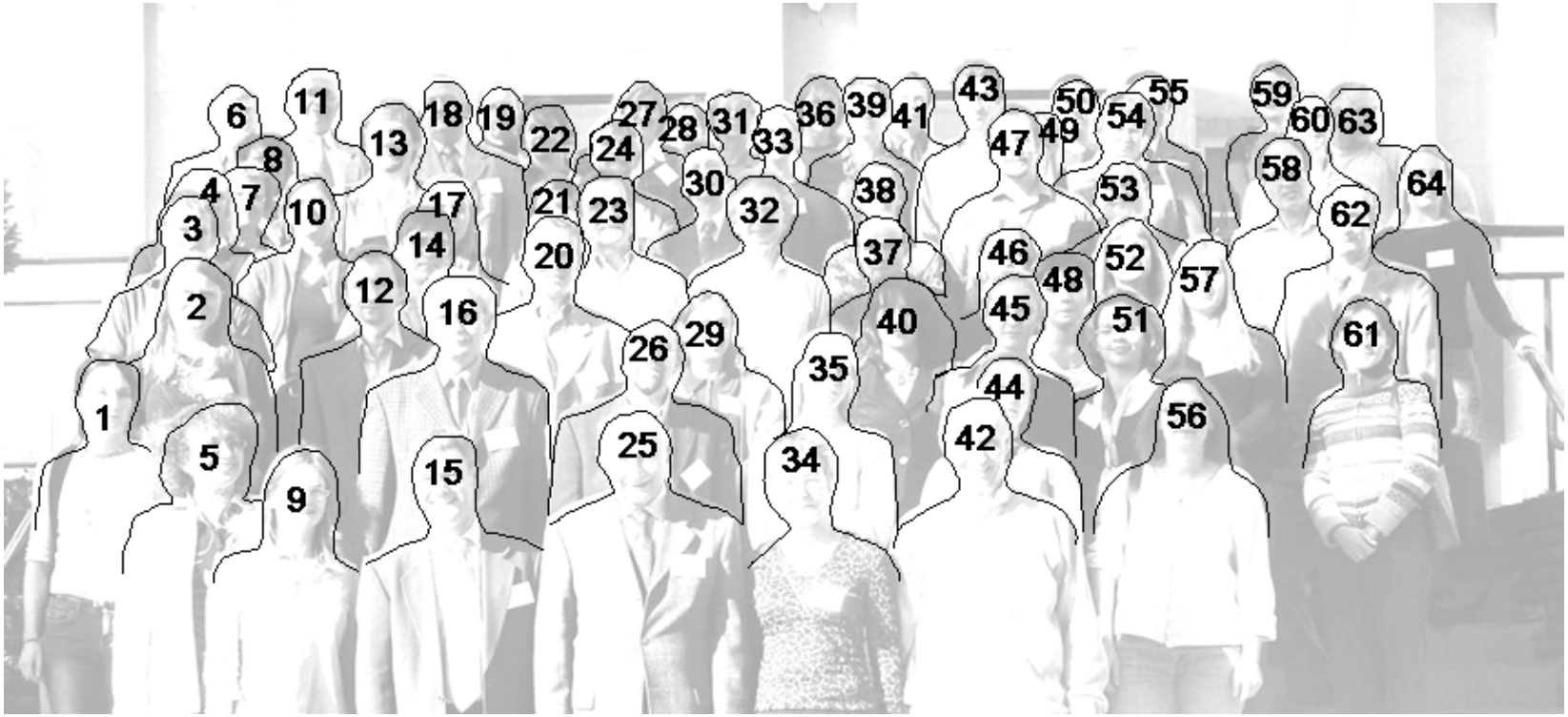
This was the first meeting convened by both the new convenors (Birger is now fully recovered from an achilles tendon rupture, which prevented his attendance at the 2004 meeting!). We gratefully acknowledge the support of the previous convenors, Volker Paul and Ingrid Williams, given to us both prior to and during this meeting. We dedicate this volume to them with gratitude for all they have done for our Working Group. We hope to see you all at CETIOM in France for the next meeting, and thank Xavier Pinochet for taking on the responsibility for local arrangements. *Á bientôt*!

Sam Cook and *Birger Koopmann*

List of Participants

Name	Organization, City/Town, Country
Aubertot, Jean-Noel	INRA/INA, Thiverval-Grignon, France
Balesdent, Marie-Hélène	INRA, Versailles, France
Baturo, Anna	Agricultural University, Bydgoszcz, Poland
Besnard, Anne-Laure	INRA, Rennes, France
Berg, Gunilla	SBA, Alnarp, Sweden
Borowczak, Paulina	Agricultural University, Poznań, Poland
Brun, Hortense	INRA, Rennes, France
Büchs, Wolfgang	BBA, Braunschweig, Germany
Cook, Sam	Rothamsted Research, Harpenden, United Kingdom
Downes, Katherine	Rothamsted Research, Harpenden, United Kingdom
Evans, Neal	Rothamsted Research, Harpenden, United Kingdom
Eynck, Christina	Georg-August University, Göttingen, Germany
Fisher, Kirsa	Georg-August University, Göttingen, Germany
Fudal, Isabelle	INRA, Versailles, France
Gladders, Peter	ADAS Boxworth, Cambridge, United Kingdom
Gutowska, Aneta	Agricultural University, Poznań, Poland
Happstadius, Ingrid	SW Seed, Svalöf, Sweden
Heimann, Stefan	Research Centre for Cultivar Testing, Słupia Wielka, Poland
Heimbach, Udo	BBA, Braunschweig, Germany
Hokkanen, Heikki	University of Helsinki, Helsinki, Finland
Hood, John	Rothamsted Research, Harpenden, United Kingdom
Hornby, Paul	Rothamsted Research, Harpenden, United Kingdom
Irzykowski, Witold	Institute of Plant Genetics, PAS, Poznań, Poland
Jajor, Ewa	Institute of Plant Protection, Poznań, Poland
Jędryczka, Małgorzata	Institute of Plant Genetics, PAS, Poznań, Poland
Kachlicki, Piotr	Institute of Plant Genetics, PAS, Poznań, Poland
Kaczmarek, Joanna	Institute of Plant Genetics, PAS, Poznań, Poland
Kałuski, Tomasz	Institute of Plant Protection, Poznań, Poland
Karolewski, Zbigniew	Agricultural University, Poznań, Poland
Kasprzyk, Idalia	University of Rzeszow, Rzeszów, Poland
Kelm, Maria	Agricultural University , Wrocław, Poland
Keunecke, Harald	Georg-August University, Göttingen, Germany
Klukowski, Zdzisław	Agricultural University, Wrocław, Poland
Koch, Simone	Georg-August University, Göttingen, Germany
Koopmann, Birger	Georg-August University, Göttingen, Germany
Korbas, Marek	Institute of Plant Protection, Poznań, Poland
Kozłowski, Jan	Institute of Plant Protection, Poznań, Poland
Krause, Ulrike	Georg-August University, Göttingen, Germany

Name	Organization, City/Town, Country
Kreye, Holger	BBA, Braunschweig, Germany
Krzymański, Jan	Institute of Plant Breeding and Acclimatization, Poznań, Poland
Lewartowska, Elżbieta	Institute of Plant Genetics, PAS, Poznań, Poland
Łukanowski, Andrzej	Agricultural University´, Bydgoszcz, Poland
Mrówczyński, Marek	Institute of Plant Protection, Poznań, Poland
Muśnicki, Czesław	Agricultural University, Poznań, Poland
Neumann, Nadine	Georg-August University, Göttingen, Germany
Odstrclova, Lenka	AGRITEC, Sumperk, Czech Republic
Olechnowicz, Julia	Institute of Plant Genetics, PAS, Poznań, Poland
Paul, Volker	University of Paderborn, Paderborn, Germany
Penaud, Anette	CETIOM, Thiverval-Grignon, France
Piliuk, Jadviha	Institute of Arable Farming and Plant Breeding, Zodino, Belarus
Pinochet, Xavier	CETIOM, Thiverval-Grignon, France
Pirie, Elizabeth	Rothamsted Research, Harpenden, United Kingdom
Plachka, Eva	OSEVA, Opava, Czech Republic
Podleśna, Anna	Institute of Soil Science and Cultivation, Pulawy, Poland
Radaunia, Uladimir	Institute of Arable Farming and Plant Breeding, Zodino, Belarus
Riediger, Nadine	Georg-August University, Göttingen, Germany
Sadowski, Czeslaw	Agricultural University, Bydgoszcz, Poland
Seta, Gustaw	Institute of Plant Protection, Sosnicowice, Poland
Skellern, Matt	Rothamsted Research, Harpenden, United Kingdom
Stachowiak, Anna	Institute of Plant Genetics, PAS, Poznań, Poland
Starzycka, Eligia	Institute of Plant Breeding and Acclimatization, Poznań, Poland
Starzycki, Michal	Institute of Plant Breeding and Acclimatization, Poznań, Poland
Tekiela, Agata	Institute of Plant Protection, Rzeszów, Poland
Twardowski, Jacek	Agricultural University, Wrocław, Poland
Ulber, Bernd	Georg-August University, Göttingen, Germany
Veromann, Eve	Institute of Plant Protection, Tartu´, Estonia
Vrbovsky, Viktor	OSEVA´, Opava, Czech Republic
Wachowiak, Henryk	Institute of Plant Protection, Poznań, Poland
Walczak, Felicyta	Institute of Plant Protection, Poznań, Poland
Wagas, Wakil	University of Arid Agriculture, Ravalpindi, Pakistan
Watts, Nigel	Rothamsted Research, Harpenden, United Kingdom
Weber, Zbigniew	Agricultural University, Poznań, Poland
Wesolowska, Ilona	Agricultural University, Poznań, Poland
Węgorek, Paweł	Institute of Plant Protection, Poznań, Poland
Williams, Ingrid	Rothamsted Research, Harpenden, United Kingdom
Wos, Henryk	Plant Breeding Strzelce, Borowo, Poland
Zary, Ewa	Agricultural University, Bydgoszcz, Poland



1 Nadine Neumann, **2** Ulrike Krause, **3** Nigel Watts, **4** Simone Koch, **5** Maria Kelm, **6** Tomasz Kaluski, **7** Gunilla Berg, **8** Joanna Cybulska-Augustyniak, **9** Sam Cook, **10** Nadine Riediger, **11** Marek Mrówczynski, **12** Zbigniew Karolewski, **13** Heikki Hokkanen, **14** Matt Skellern, **15** Birger Koopmann, **16** Zdzislaw Klukowski, **17** Joanna Kaczmarek, **18** Stefan Heimann, **19** Jan Pszczola, **20** Holger Kreye, **21** Ewa Jajor, **22** Eva Plachka, **23** Bernd Ulber, **24** Marek Korbas, **25** Jan Krzymanski, **26** Wolfgang Büchs, **27** Anna Podlesna, **28** Lenka Odstrcilova, **29** Annette Penaud, **30** Czeslaw Sadowski, **31** Elzbieta Lewartowska, **32** Udo Heimbach, **33** Christina Eynck, **34** Ingrid Williams, **35** Eve Veromann, **36** Hortense Brun, **37** Xavier Pinochet, **38** Marie-Hélène Balesdent, **39** Kirsa Fischer, **40** Anna Baturo, **41** Viktor Vrbovsky, **42** Volker Paul, **43** Harald Keunecke, **44** Elisabeth Pirie, **45** Ewa Zary, **46** Anne-Laure Besnard, **47** Paul Hornby, **48** Ilona Wesolowska, **49** Ingrid Happstadius, **50** John Hood, **51** Anna Stachowiak, **52** Paulina Borowczak, **53** Jean-Noel Aubertot, **54** Jadviha Piluk, **55** Uladimir Radaunia, **56** Malgorzata Jedryczka, **57** Aneta Gutowska, **58** Peter Gladders, **59** Jenna Stonard, **60** Neal Evans, **61** Idalia Kasprzyk, **62** Witold Irzykowski, **63** Piotr Kachlicki, **64** Katherine Downes

Contents

Effects of production measures on plant health

ENTOMOLOGY PAPERS

Pest damage to oilseeds – Problems

Pest damage to oilseeds – Solutions (i) Insecticides and issues regarding insecticide resistance

Pest damage to oilseeds – Solutions (ii) IPM - Manipulating pest behaviour

Pest damage to oilseeds – Solutions (iii) IPM – Predators and parasitoids

Pest damage to oilseeds – Techniques for developing solutions

PATHOLOGY PAPERS

Light leaf spot

Blackleg, stem canker

Sclerotinia stem rot

Verticillium

Other diseases

GENERAL PAPERS

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 3-5

History, aims, and achievements of the IOBC/wprs Working Group on Integrated Control in Oilseed Crops from 1979-2006

Birger Koopmann[1], Samantha M Cook[2], Ingrid H. Williams[2] and Volker H. Paul[3]
[1] Georg-August University, Göttingen, Department of Crop Sciences, Division of Plant Pathology and Plant Protection, Germany; [2]Plant & Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ. UK; [3]University of Applied Sciences, Paderborn, Germany

The history of the working group 'Integrated Control in Oilseed Crops' (ICOC) goes back to the seventies of the last century. In 1979, a study group split away from the working group '*Integrated Control in Brassicas*', led by Tom Coaker, which focussed mainly on the cabbage root fly (*Delia radicum*). Bent Bromand, a Danish entomologist (Danish Research Centre for Plant Protection), tried together with his Swedish colleague, Christer Nilsson (Swedish University of Agriculture), to establish a separate working group on `*Integrated Control in Oilseed Rape´*. This working group was founded in 1982. In the meantime (1979-1982), it had the status of a study group. Both study and working group were led by Bent Bromand. During 1979-1988, Bent Bromand organized four workshops of the group (Table 1). He resigned his convenorship in 1988 and was followed by Volker Paul (University of Paderborn, Germany). The new convenor Volker Paul was in his honorary position between 1988 and 2003. He was assisted by Ingrid Williams (Rothamsted Research, United Kingdom), who led the entomology subgroup, which was established during this time. Ingrid Williams and Volker Paul organized twelve group/subgroup meetings within this time period (Table 1). In 1990, the group decided to expand its activities from oilseed rape to other oilseed crops and to change its title to the '*Working Group on Integrated Control in Oilseed Crops*'. During 1988-2003 Volker Paul and Ingrid Williams edited seven IOBC/wprs Bulletins together with assisting colleagues. They resigned their convenorships in 2003 (Volker Paul) and 2004 (Ingrid Williams), respectively. They were followed by Birger Koopmann (University of Göttingen, Germany) and Samantha Cook (Rothamsted Research, United Kingdom), elected in 2003 and 2004, respectively. During this time, two workshops were organized and two IOBC/wprs Bulletin were published (including this issue).

Working group aims

The working group was established to encourage and coordinate research on integrated disease and pest management systems in oilseed production. Through its regular meetings, it is also intended to serve as a platform to gather and exchange fundamental knowledge and expertise on diseases and pests. Major areas of focus for the group have been:

- Occurrence and distribution of diseases and pests (monitoring work)
- Integrated pest and disease management
- Establishment of damage thresholds
- Development of decision support systems
- Disease resistance
- Use of trap crops for pest control
- Biology of pest and beneficial insects

- Biological control (predators, parasitoids and antagonists)
- Seed pathology in oilseed crops
- Gene technology in oilseed crops – Significance, economics and environment

Recently, the group has encouraged participation of central European scientists at its meetings. This was initiated by organizing meetings in Poznan (1997, 2005) and Prague (1999). The success of this integration is documented by the high proportion of Central European colleagues who attended the last WG-ICOC meeting. From a total number of 76 who attended, 55% were from central European contries, including Belarus, Czech Republic, Estonia and Poland. Scientists from these countries are now attending our meetings regularly and are actively contributing to our deliberations and collaborative projects.

Table 1. Chronicle of ICOC-Working Group Meetings

Year	Location	Country	Convenor/s*	Remarks
1982	Paris	France	BB	Status: Study Group
1984	Copenhagen	Denmark	BB	Status: Working Group, WG name: Integrated Control in Oilseed Rape
1986	Braunschweig	Germany	BB	
1988	Malmö	Sweden	BB	
1990	Rothamsted	United Kingdom		Change of WG name to *Integrated Control in Oilseed Crops*
1991	Braunschweig	Germany	VP, IW	Pathology (P) & Entomology (E) subgroup meetings
1992	Le Rheu	France	VP, IW	P & E subgroup meetings
1993	Cambridge	United Kingdom	VP, IW	P & E subgroup meetings
1993	Paderborn	Germany	VP, IW	P & E subgroup meetings
1994	Zürich	Switzerland	VP, IW	P & E subgroup meetings
1995	Cambridge	United Kingdom	VP, IW	P & E subgroup meetings
	Informal meeting and contact meeting with non-IOBC/wprs members at the 9th International Rapeseed Congress of the GCIRC			
1995	Paderborn	Germany	VP, IW	P & E subgroup meetings
1997	Poznań	Poland	VP, IW	P & E subgroup meetings
1999	Prague	Czech Republic	VP, IW	P & E subgroup meetings
2001	Soest	Germany	VP, IW	P & E subgroup meetings
2003	Copenhagen	Denmark	VP, IW	P & E subgroup meetings
	Informal meeting and contact meeting with non-IOBC/wprs members at the 13th International Rapeseed Congress of the GCIRC			
2004	Harpenden	United Kingdom	BK, IW	P & E subgroup meetings
2005	Poznań	Poland	BK, SC	P & E subgroup meetings *Supported by the EU project PAGEN*

* Convenor abbreviations: BB – *Bent Bromand*, BK – *-Birger Koopmann*, IW – *Ingrid Williams*, SC – *Samantha Cook*, VP – *Volker Paul*

Achievements and publications

The emphasis of the working group has always been the networking of scientists working towards improved integrated pest and disease management of oilseeds, particularly the oilseed rape crop. Collaborative field experiments have been conducted on specific questions and joint projects established. Among these are included four large international projects funded by the European Union. These projects were conceived, are coordinated and executed mainly by members of the working group. These EU-Projects are:BORIS

- IMASCORE
- MASTER (http://www.rothamsted.bbsrc.ac.uk/pie/master/master.htm)
- SECURE (http://www.secure.rothamsted.ac.uk/)

Further information about the projects can be found on their respective websites.

In 2004, we decided to establish a Working Group webpage, which is intended to announce meetings, meeting contributions, reports of past meetings and news. Information is available under: http://wwwuser.gwdg.de/~instphyt/app/koopmann/eng-dateien/iobc2004-bulletin.htm.

Papers presented at Working Group meetings are published in the IOBC/wprs Bulletin series. Altogether, the group has published 9 Bulletins comprising a total number of 2277 pages (Table 2). Also, a book was published in 2003 as a result of information gathered as part of the EU-funded BORIS Concerted Action. This was entitled: *Biocontrol of oilseed rape pests* and edited by DV Alford, Oxford, UK: Blackwell Publishing. 355 pp.

Table 2. Chronicle of IOBC Bulletins from the ICOC-Working Group Meetings

Publication	Year	Number of pages
IOBC/ wprs Bulletin Vol. 13 (04)	1990	308 pp.
IOBC/ wprs Bulletin Vol. 14 (06)	1991	308 pp.
IOBC/ wprs Bulletin Vol. 16 (09)	1993	234 pp.
IOBC/ wprs Bulletin Vol. 18 (04)	1995	133 pp.
IOBC/ wprs Bulletin Vol. 21 (05)	1998	239 pp.
IOBC/ wprs Bulletin Vol. 23 (06)	2000	211 pp.
IOBC/ wprs Bulletin Vol. 25 (02)	2002	151 pp.
IOBC/ wprs Bulletin Vol. 27 (10)	2004	302 pp.
IOBC/ wprs Bulletin Vol. 29 (this issue)	2006	391 pp.

Future

We have our next meeting in France, which will be organized by CETIOM colleagues in 2007/2008. We also look forward to developing new work programmes and strengthening links between the pathology and entomology sub groups in the future. One step in this direction may result from a potential case study in oilseed rape under the new European Network of Excellence 'ENDURE' (European Network for the DURable Exploitation of crop protection strategies) which involves institute partners of several members of this working group.

Production of oilseeds: Global perspectives

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 9-15

Oilseed Crops in Poland – Past and perspectives

Jan Krzymański
Institute of Plant Breeding and Acclimatisation, Oil Crop Department, 36 Strzeszynska Street, 60-479 Poznan, Poland

Abstract: Systematic study on oilseed crops after 2nd World War started in Poland in 1950, when the Oil Crop Department in Poznan was established in the Plant Breeding and Acclimatisation Institute. As broad a spectrum of species were examined as possible for oilseed crops for Poland. Possibilities of acclimatisation and expected economical values were investigated. Then the most promising species were chosen and research work and breeding were limited to the following crops:

- Winter and spring oilseed rape (*Brassica napus*), winter oilseed turnip rape (*Brassica rapa*) and sunflower (*Helianthus annuus*) – oils for edible purposes,
- Linseed (*Linum usitatissimum*) and false flax (*Camelina sativa*),
- Poppy (*Papaver somniferum*) – seed for nutritional purposes and raw material for pharmaceutical industry,
- Yellow mustard (*Sinapis alba*) - as a spice and phytosanitary aftercrop.

The work done up to the present time demonstrates that oilseed rape is the oil crop which performs best of all in the agroclimatic conditions of Poland. Research on the genetics and the biochemistry of *B. napus* formed the base for breeding of new, so called double low varieties characterised by a lack of erucic acid in oil and very low levels of glucosinolates in meal. Changeover to these new varieties was done in Poland in years 1985 – 1990. Now the changeover to hybrid varieties has subsequently taken place.

Direction of future research and breeding work:

- Adaptation of fatty acid composition to better fit the different oil uses;
- Increasing of fat and protein contents in seeds;
- Improvement of meal by further decreasing the content of antinutritive components like: alkenyl glucosinolates, dietary fiber, sinapine, polyphenols, phytin;
- Investigate sources of resistance or tolerance to diseases and to pests and to stress conditions (winterhardiness, drought resistance);
- Improvement of agronomic value e.g. yielding ability, resistance to lodging, resistance to shattering;
- Development and use of new method like tissue culture, doubled haploid production, protoplast fusion, vegetative propagation, interspecific crosses, embryo culture,
- Production of better hybrids using CMS or SI system
- Marker assisted selection, estimation of genetic distance;
- Improvement of oil stability – tocopherol content.

An increase of rapeseed production is expected in Poland in next years which highlights the importance of continued breeding work

Key words: Oilseed crops, oilseed rape, sunflower, mustard, linseed, poppy, research, breeding, cultivation, pests, diseases

History

The systematic study of oilseed crops after 2nd World War started in Poland in 1950, when the Oil Crop Department in Poznan was established in the Plant Breeding and Acclimatisation Institute.

At the beginning, as broad a spectrum of species were examined as possible with respect to prospective oilseed crops for Poland. Possibilities of introduction and acclimatisation were investigated. Also quality of oil and expected economical potential were taken into consideration.

The following species were examined:

- Winter and spring oilseed rape (*Brassica napus*), winter and spring turnip rape (*B. rapa*),
- Black, brown and yellow mustards (*Brassica nigra, Brassica juncea, Sinapis alba*)
- Oilseed radish (*Raphanus sativus*), crambe (*Crambe abyssinica*), eruca (*Eruca sativa*),
- Linseed and flax (*Linum usitatissimum*), winter and spring false flax (*Camelina sativa*),
- Hemp (*Cannabis sativa*), perilla (*Perilla frutescens*),
- Sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), niger (*Guizotia abyssinica*),
- Soybean (*Glycine max*), peanut (*Arachis hypogaea*),
- Castor (*Ricinus communis*),
- Pumpkin (*Cucurbita pepo*), squash *Cucurbita maxima*),
- Poppy (*Papaver somniferum*), sesame (*Sesamum indicum*),

After this preliminary study the most suitable and promising species for Polish conditions were chosen for further study. Over the following years, research and breeding was limited to the following crops:

- Winter and spring oilseed rape (*Brassica napus*), winter oilseed turnip rape (*Brassica rapa*) and sunflower (Helianthus annuus) – supplying oils for edible purposes,
- Linseed (*Linum usitatissimum*) and false flax (*Camelina sativa*) – supplying drying oils,
- Poppy (*Papaver somniferum*) – producing seed for nutritional purposes and capsules a raw material for pharmaceutical industry,
- Yellow mustard (*Sinapis alba*) - used as a spice and as phytosanitary aftercrop.

In the 1960’s, it was expected that demand on drying oils would decrease as a result of the introduction of synthetic polymers, so work on linseed and false flax was very limited. This forecast was proved to be premature and it was necessary to recommence research work and breeding on linseed. Several new varieties also with yellow seeds were bred as a result of this work.

Polish varieties of yellow mustard cover demand for spices and the needs of pharmaceutical industry. A considerable quantity of seeds is used to sow phytosanitary aftercrops. However, yellow mustard is more resistant to drought than spring oilseed rape and may be better oilseed crop for Poland. Research and breeding works are now being done to obtain plants with reduced levels of erucic acid and glucosinolates. New lines of desired quality have been selected and double low (“00”) varieties of yellow mustard will be bred in the near future.

There was an increase of drug abuse in Poland at the beginning of eighties and it was necessary to start work to obtain new varieties of poppy with substantially lowered morphine content. Several new varieties practically without morphine were bred and introduced to cultivation. These varieties have different shape or colour of flowers as a marker.

Sunflower was considered as a promising oil crop for Poland in 1960’s. As this species had the same seed yield as oilseed rape but better oil quality. However, sunflower ripens in September in Poland but the heads do not dry because of wet weather conditions during this

month. This results in fungal attack which reduces both yield and seed quality. Improved harvesting method designed for Polish conditions have not as yet been developed.

Winter oilseed turnip rape was cultivated in North Poland as this species had better winter hardiness. However, it was yielding about 30 per cent lower than winter oilseed rape so cultivation of turnip rape was drastically reduced when new oilseed rape varieties with improved winter hardiness were bred. Also better cultivation practices (sowing time, limited nitrogen fertilisation in autumn) improved winter hardiness of oilseed rape.

The work done up to the present time demonstrates that oilseed rape is the oil bearing crop which performs best of all under Polish climatic conditions, similarly as seen in other European Countries lying in the colder part of temperate climate. The lower yielding spring form is cultivated only on a limited area, mainly as a replacement crop in the case of substantial winter damage on winter oilseed rape crops.

Oilseed rape belongs to the Brassica family. High erucic acid content in seed oil and glucosinolate content in all tissues are characteristic for this family. Erucic acid content in oils from traditional varieties was high – about 50 per cent. Many experiments have shown that a high content of erucic acid in the diet not only represses body weight gain, but can also be harmful for the heart, liver, adrenal gland and spleen. Only after elimination of erucic acid from rapeseed did rapeseed oil become edible.

Rapeseed meal, which remains after seed oil extraction, contains between 36 and 41 per cent protein on a dry matter basis. This protein has a well balanced amino acid composition, better than proteins from soybean or casein. Rapeseed meal contains more sulphur amino acids. However, the feeding value of meal from traditional oilseed rape varieties has been limited by the presence of sulphur compounds called glucosinolates.

When glucosinolates are hydrolysed by the myrosinase enzyme, also present in seeds, isothiocyanates, thiocyanates and nitrile are released. These compounds when feed to animals reduce palatability, adversely affect iodine uptake of the thyroid gland resulting in metabolic disorders, poor feed efficiencies and reduced weight gains. Seed of traditional varieties contained 110 to 150 μmoles of the aliphatic glucosinolates per gram of oil.

Both erucic acid and glucosinolates were eliminated by recombination breeding using natural genetic sources of desirable genes. These sources were found in spring oilseed rapes that had been cultivated for a long time in Poland. The variety Bronowski was identified as having only 10 to 12 μmoles of aliphatic glucosinolates and also only 7 per cent erucic acid in seed oil. Bronowski was the only source of very low glucosinolate content used world wide in breeding programs. The variety was bred in Poland and licensed in 1955. Subsequently, zero erucic lines were found in Liho variety, selected in Germany from materials collected in Poland.

Inheritance study indicated that the amount of erucic acid is controlled by genotype of the embryo through two pairs of genes in an additive manner. Glucosinolate content is controlled through the genotype of the maternal plant as a polygenic trait. Both traits were incorporated to adapted varieties in backcross programs, or by recombination breeding. This genetic biochemistry formed the base for breeding of new, so called double low varieties characterised by lack of erucic acid in oil and very low level of glucosinolates in meal.

Removal of erucic acid and glucosinolates was connected with loss of plant vigour and winter hardiness. Many years of breeding work was necessary to obtain productive varieties adapted to Polish conditions. The first Polish double low oilseed rape variety Jantar was licensed in 1985. A list of Polish double low winter oilseed rape is given in Table 1.

Table 1. List of Polish varieties of winter oilseed rape (year of licensing)

Line varieties:		Hybryd varieties:	
Jantar	(1985)		
Bolko	(1989)		
Mar	(1991)		
Leo	(1993)		
Polo	(1993)		
Marita	(1994)		
Kana	(1997)		
Gara	(1999)		
Batory	(2001)	Kaszub	(2001)
Bazyl	(2001)	Mazur	(2001)
Bosman	(2002)	Lubusz	(2002)
Bojan	(2003)	Pomorzanin	(2002)

The licensing of Jantar allowed us to begin the changeover to double low ("00") varieties, which was done in Poland between 1985-1990 (Table 2). More recently there has been a changeover to hybrid varieties.

Table 2. Introduction of double low rapeseed varieties to cultivation in Poland

Harvest year	Total area of cultivation [1000 ha]	Yielding area under double low rapeseed [1000 ha]	Double low rapeseed [per cent]
1985	467.0	3.39	0.7
1986	515.0	18.05	3.5
1987	499.0	41.65	8.3
1988	470.0	97.51	10.7
1989	570.0	236.72	41.5
1990	500.0	490.0	98.0
1991	467.0	457.0	97.8

Breeding of double low varieties of winter oilseed rape and their introduction to cultivation followed a huge team effort by many people. Work began in 1968. The work began with genetic and biochemistry and then expanded to include breeding and cultivation research before finishing with animal feeding experiments and oil mill technology trials. Through all of this work, farmers obtained not only the seed of new varieties but also instructions describing the cultivation manner to be used and also how to use the new rapeseed meal in animal feed mixtures.

Data on oilseed rape production in Poland

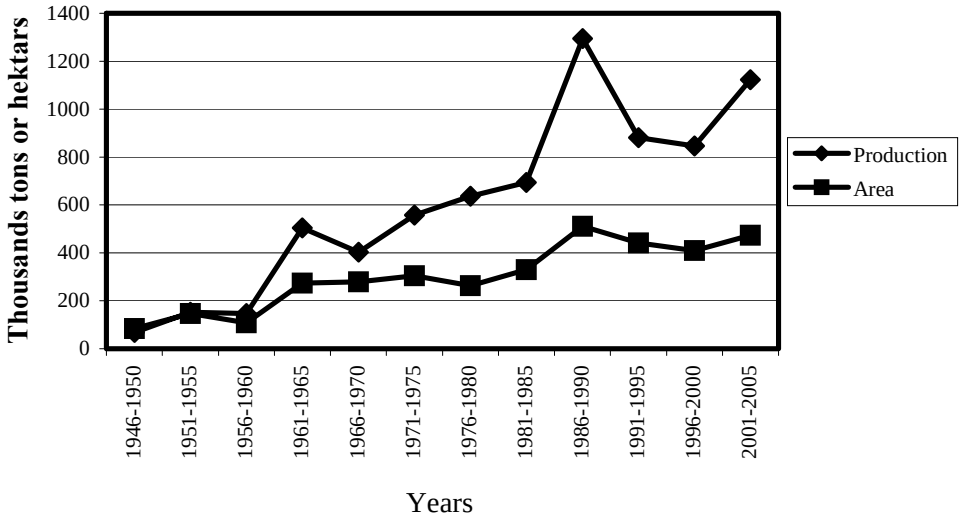


Figure 1. Cultivation area and production of oilseed rape in Poland

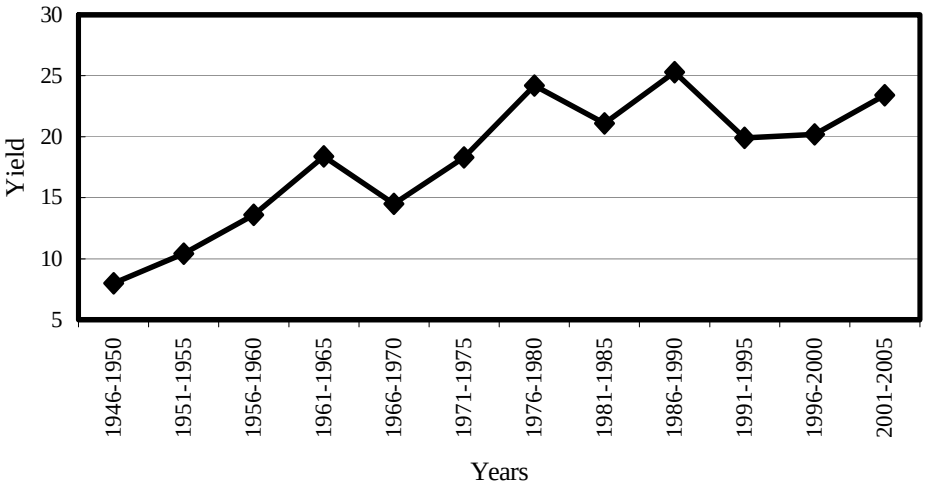


Figure 2. Seed yield of oilseed rape in Poland (dt/ha)

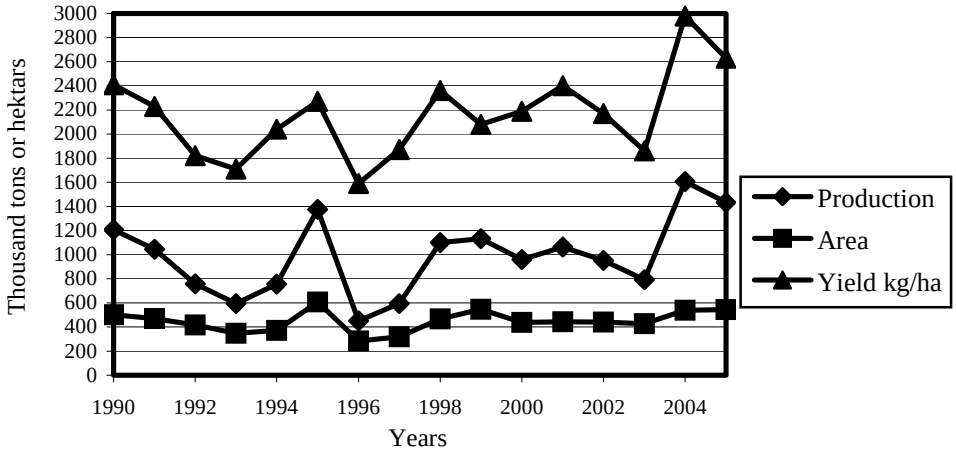


Figure 3. Results of oilseed rape cultivation during 1990-2005 in Poland

Current direction of Polish oilseed rape breeding

- High and constant seed yield
- High oil yield and quality
- Meal quality
- Resistance or tolerance to diseases
- Tolerance to pest
- Resistance to stresses
- Hybrid variety development

Pests and diseases

The most important pests of oilseed rape in Poland:

- Stem weevils (*Ceutorhynchus napi, Ceutorhynchus quadridens*)
- Pod (siliquae) pests (*Ceutorhynchus assimilis, Dasyneura brassicae*)
- Pollen beetle (*Meligethes aeneus*)

The following pests can be problematic in some years:

Ceutorhynchus pleurostigma, Ceutorhynchus sulcicollis, Ceutorhynchus picitarsis, Psylliodes chrysocephala, Agrotinae, *Brevicoryne brassicae, Phorbia brassicae, Deroceras reticulatum.*

Important fungal diseases of oilseed rape in Poland:

- *Leptosphaeria maculans/Leptosphaeria biglobosa,*
- *Sclerotinia sclerotiorum,*
- *Alternaria spp.,*
- *Botrytis cinerea,*
- *Pyrenopeziza brassicae* (*Cylindrosporium concentricum*),
- *Verticillium spp.,*
- *Peronospora parasitica,*
- *Erysiphe cruciferarum,*
- *Pseudocercosporella capsellae*

These fungi are given in order of their importance. Leptosphaeria/Phoma is the most frequent pathogen encountered in the north of Poland but Sclerotinia is more prevalent in southern areas.

Future direction for research and breeding programs

- Adaptation of fatty acid composition to better fit different oil uses;
- Increase fat and protein contents in seeds;
- Improvement of meal by further decreasing the content of antinutritive components like alkenyl glucosinolates, dietary fibre, sinapine, polyphenols and phytin;
- Investigate sources of resistance or tolerance to diseases and to pests and to stress conditions (winterhardiness, drought resistance);
- Improvement of agronomic value e.g. yielding ability, resistance to lodging, resistance to shattering;
- Development and use of new method like tissue culture, doubled haploid production, protoplast fusion, vegetative propagation, interspecific crosses, embryo culture,

- Production of new hybrids using CMS or SI system
- Marker assisted selection, estimation of genetic distance;
- Improvement of oil stability – tocopherol content.

Conclusion

A sharp increase in the area of rapeseed production in Poland is expected over the next few years. It is mainly due to increased non-food use of rapeseed oil, mainly as a raw material for biofuel and lubricants.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 17-27

Integrated pest management in oilseed crops in Pakistan

Waqas Wakil[1] and Shahbaz Talib Sahi[2]
[1] *Department of Entomology, University of Arid Agriculture, Rawalpindi, Pakistan.*
e-mail: waqaswakeel@hotmail.com;
[2] *Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan*

Abstract: In Pakistan the oilseed crops are seed cotton, rapeseed, sunflower, sesame seed, groundnut, soybean, linseed, castor bean, coconut and safflower. Over the last half century (from 1961 to 2004), the area under oilseed crops has increased by about 100% i.e. from 1,963,980 hectares in 1961 to 3,964,266 hectares in 2004. Although the yield of oilseed crops is quite low as compared to many other countries of the world, it has increased by about 270% (from 1054 kg/ha in 1961 to 2951 kg/ha in 2004), during the period under discussion. Abiotic and biotic factors responsible for lower yields in Pakistan are discussed, with particular emphasis on pests. Different approaches for integrated pest management in practice in Pakistan are discussed in this paper.

Key words: Integrated pest management, oilseed crops, Pakistan

Introduction

Pakistan, being predominantly an agricultural country, contributes almost 25 % to Gross Domestic Production (GDP) and sustenance to about 70% of the population in addition to providing a source of employment for nearly 50% of the total labor force. Contribution of the agricultural sector to foreign exchange earnings is about 80%, through the export of commodities such as cotton products and rice (Anonymous, 2003).

The main crops under cultivation in Pakistan are wheat, cotton, rice and sugarcane. Oilseed crops sown in Pakistan are mainly of two types: traditional (seed cotton, rapeseed, sunflower, groundnut and sesame) and non-traditional (safflower, soybean, linseed, castor bean and coconut). However, the area under all these crops has always been very low as compared to other crops. Traditional oilseed crops occupy about 80% of the total area under oilseed crops (Anonymous, 2004). Among the traditional oilseed crops, in 2004, cottonseed was ranked first on area grown, followed by rapeseed, sunflower, sesame and groundnut (Figure 1a) while in the case of nontraditional oilseed crops, the area dedicated to soybean is greatest, followed by linseed, castor bean, coconut and safflower in 2004 (Figure 1b). In 1961 the area under all these crops was quite low but it has increased substantially over time. Yield of these crops in Pakistan has been quite low compared with other countries yet it has increased by about 270 % over the last half century (FAO, 2004). In the case of traditional crops the yield of sunflower, rapeseed, sesame and cotton increased significantly from 1961 to 2004 while that of groundnut decreased over this period (Figure 2a). To discuss the reason for this unusual trend is beyond the scope of this paper. However, the ~900% increase in the area under groundnut between 1961 and 2004 indicates that perhaps the majority of this area consists of marginal, poor land which has reduced the overall per hectare yield, despite the introduction of high-yielding varieties. In the case of nontraditional crops, yield in all the crops has been quite unstable during the period under report (Figure 2b). The main reason for the fluctuations may be that these crops have never been taken seriously by the government as

well as the farmers. Additionally, fluctuations in yield may also be attributed to certain abiotic and biotic stresses. A general discussion of such factors is included in the section on the limitations for successful cultivation of oilseed crops in Pakistan.

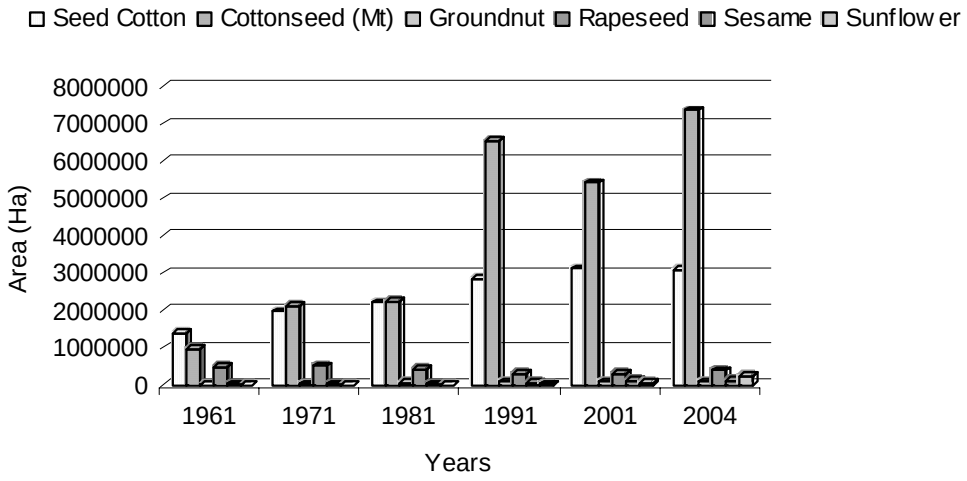


Figure 1a. Area (ha) under different traditional oilseed crops in Pakistan

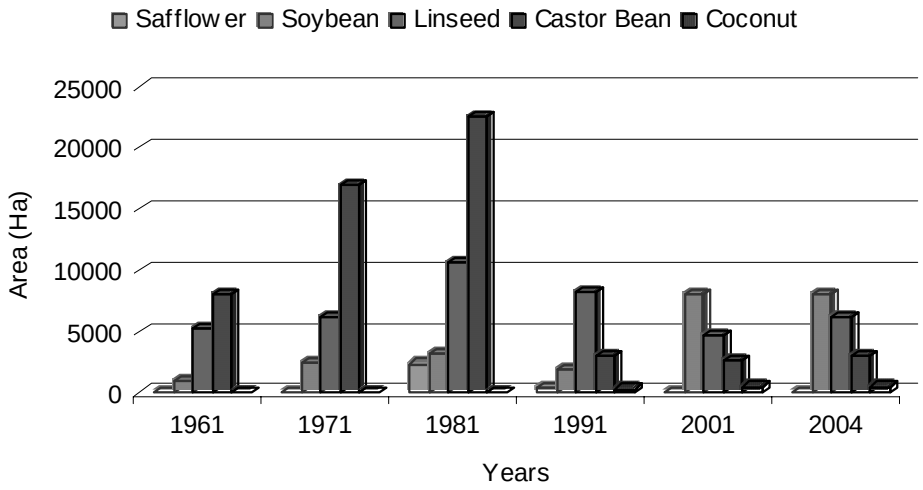


Figure 1b. Area (ha) under different non-traditional oilseed crops in Pakistan

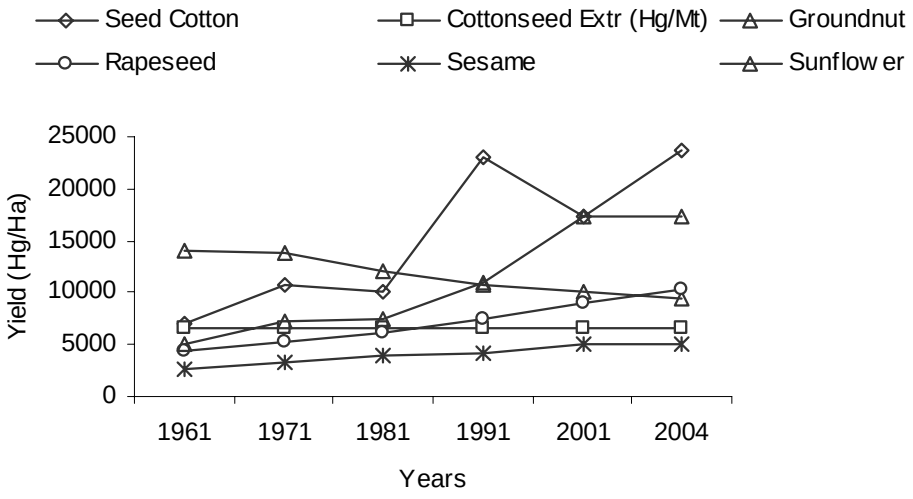


Figure 2a. Yield (Hg/Ha) of different traditional oilseed crops in Pakistan

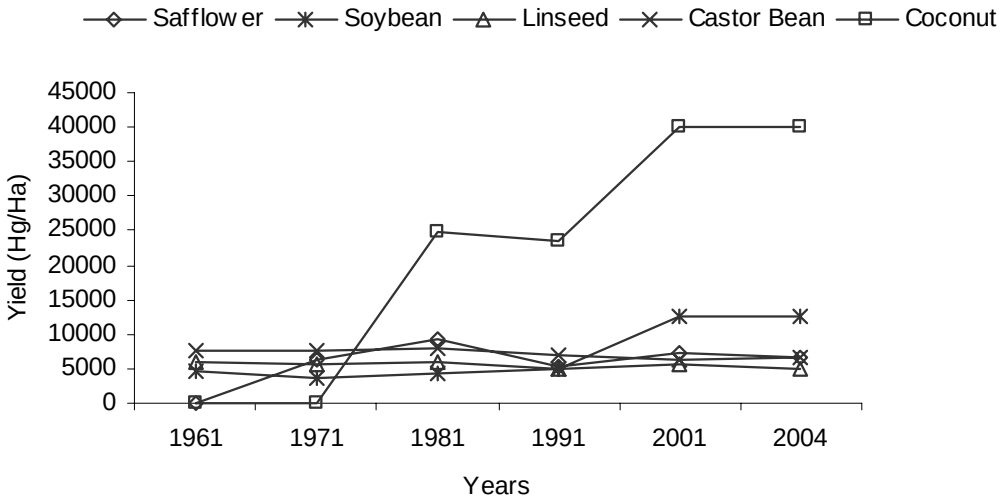


Figure 2b. Yield (Hg/Ha) of different non-traditional oilseed crops in Pakistan

The low yields of oilseed crops in Pakistan result in a major dependence on the import of edible oil; the local production has been sufficient to meet only 20-36 percent of the country's requirement over the last 15 years (Anonymous, 2003). Hence, there is a need to increase the yield by properly addressing all the factors responsible for low yields. A general overview regarding the oilseed crops grown in Pakistan and the important pests responsible for their low yield is given below.

Traditional Oilseed Crops

Cotton Seed

Cotton, in addition to being a fiber crop, is the major source of edible oil in Pakistan, accounting for more than 60% of the indigenous vegetable oil production (Anonymous, 2003).

About 150 pests have been reported to feed on cotton (Shabbir, 1973). Of these, whitefly (*Bemisia tabaci*), cotton jassid (*Empoasca* sp.), thrips (*Thrips tabaci*), pink bollworm (*Pectinophora gossypiella*), spiny bollworm (*Earis insulana*), spotted bollworm (*Earias vittella*) and African (American) bollworm (*Helicoverpa armigera*) are major pests of cotton in Pakistan.

Groundnut

Groundnut (*Arachis hypogaea* L.) is also known as peanut or earthnut. Local names for groundnut include *Moong phali* in Urdu and Punjabi, *Moom phali* in Pashto, and *Bohi monga* in Sindhi. Groundnut was not grown on a commercial scale in Pakistan before Partition (1947). Afterwards, its cultivation on a commercial scale was introduced in both Punjab and Sindh provinces. However, its cultivation was mainly concentrated (more than 90%) in Punjab province due to the availability of more suitable areas. Only ~7% of area is shared by North West Frontier Province (NWFP) and only ~1% by Sindh province. The groundnut production in Pakistan is all locally consumed as roasted kernels. Groundnut kernels contain 50% oil, which is considered very good cooking oil. The resulting meal is rich in protein (55%) and can be used as food or feed. The present yield of groundnut in the country is only 28% of the potential yield which can be increased by two to three times by adopting improved production practices, bringing more of the potential area under groundnut cultivation and by strengthening the plant protection measures.

The crop is attacked by termites, jassids (*Empoasca* sp.), and whitefly (*Bemisia tabaci*). Serious damage is caused by rats; it is estimated that 25-33% of the crop is lost due to rats. Wild boars and porcupines are also potentially damaging.

Rapeseed

Rapeseed is commonly known as *Sarsoon*. The most common types grown in Pakistan are *Brassica rapa (campestris)*, *Brassica napus*, *Brassica juncea* and *Eruca sativa*. Generally, the term “rapeseed” refers to *Brassica rapa* and *Brassica napus*. Most of the local types “desi” belong to the *Brassica rapa* group. This group comprises *Toria*, Yellow Sarsoon. *Brassica napus* is generally known as *Gobi Sarsoon* or *Japani Sarsoon*. The name ‘mustard’ is of European origin and includes *Brassica juncea, Raya* or ‘Indian mustard’, *Brassica carinata (abesina raya* or *gulabi sarsoon*), and *Eruca sativa* or *Taramira*. There are some other types which are of no economic importance in Pakistan, for example *Brassica nigra*, *Brassica tourne fortii* and *Sinapsis alba*.

As in case of previous crops, the maximum area under rapeseed in Pakistan (50.5%) is occupied by the Punjab province followed by Sindh (26.3%), North West Frontier Province (NWFP) (12.3%) and Balochistan province (10.9%). The major insect pests of rapeseed are the painted bug (*Bagrada picta*) and aphids (*Aphis gossypii*). Both the adults and nymphs of these insects suck cell sap from leaves, flowers, and pods.

Sesame

Sesame (*Sesamum indicum* L.) is called *Til* in Urdu and Punjabi, *Tir* in Sindhi, *Konzola* in Pashto, and *Kunjit* in Balochi. It is an annual minor kharif (summer) oilseed crop.

Out of the total area under sesame cultivation in Pakistan, ~95% is in Punjab province, ~ 2% in Sindh province, 2% in Balochistan province, and only ~1% in North West Frontier Province (NWFP). Sesame is mainly used in confectionery products but its use as an oilseed crop has not yet been fully explored. Sesame produces high-quality colorless and odorless edible oil, which is used for cooking and salad and for improving the quality of vegetable oils. Unlike other edible oils, sesame oil does not get rancid. It is also used in medicine and in the

preparation of high-quality soaps. Sesame contains 22% protein, while sesame cake has 42% protein and is an excellent feed for milk cattle and laying hens (Khan and Sheikh, 1985).

Sesame is attacked by a variety of insects; the most harmful being til leaf roller (*Antigastra catalaunallis*) but it is seldom economical to use insecticides for their control. Instead, cultural methods are generally employed to offset the losses caused by this insect. In addition, jassids (*Empoasca* sp.) and whiteflies (*Bemisia tabaci*) also need to be controlled since they carry the viruses which cause phyllody and leaf-curl diseases.

Sunflower

The sunflower (*Helianthus annuus* L.) is locally known as *Surajmukhi*. In Pakistan, sunflower was first introduced as an oilseed crop rather late (in the 1960's). The sunflower production gained momentum slowly in the beginning but at present it occupies a substantial area. The major production occurs in the Punjab province (about 75%) followed by Sindh province (~20%) and the rest is occupied by North West Frontier Province (NWFP). One of the major reasons for the increased area under sunflower cultivation is that no major crop has been disturbed or replaced by its cultivation as it can be accommodated between the two major crops in a cotton and rice-based cropping rotation, or on summer-fallow lands. Moreover, sunflower has shown good intercropping compatibility with other crops. The extraction of oil and processing by the present industry in the country and even at the village level by the farmer has further increased the prospects for sunflower production.

Sunflower is generally infested by armyworms (*Spodoptera litura*), cutworms (*Agrotis* spp.), hairy caterpillars (*Euproctis lunata*) and headworm (*Helicoverpa armigera*). These insects attack the plants at the larval stage and feed on leaves, stems, and young buds. Young seedlings are also attacked by whitefly and aphids.

Non-Traditional Crops

Castor bean

Castor (*Ricinus communis* L.) is locally known as *Arind* or *Arindi*. The plants may be short-lived dwarf annuals or large perennials which develop into a bush. Tall types are 6-10 m and short types are 2-3 m in height. Both the main stem and the side branches originating from the internodes terminate in a raceme. The types available in Pakistan have 15-20 internodes (Khan *et al.*, 1986). In Pakistan, the area under castor has been continuously decreasing since 1978.

Castor oil is used in the manufacture of paints and varnishes, detergents, synthetic resins and fibers, soap, ointments, cosmetics, hairdressings, brake fluids, and printing inks. It is also used as a purgative and laxative in human as well as veterinary medicine. Silkworms can be reared on green castor leaves. Castor seed protein is used in the manufacture of distempers, oil-bound water paints, and adhesives for wood. Castor cake is mainly used as manure, since the toxin ricin is insoluble in oil and remains in the oilcake, which cannot be fed to animals.

The main insect pests of castor are semilooper (*Achaea janata*), jassid (*Empoasca* sp.), hairy caterpillar (*Euproctis lunata*), and capsule borer (*Dichorochis punctiferatis*).

Coconut

In Pakistan, coconut (*Cocos nucifera* L.) has been grown on a domestic scale for many years. The first large scale planting was done in 1957. During 1977, about 15,000 seed nuts were imported from Sri Lanka and Malaysia, which were distributed to private farmers for planting on an area of 81 hectares. In 1987, the Coconut Forest Division was established at Thatta, which imported 100,000 seed nuts and planted them on an area of 4047 hectares (Anonymous, 1995). Coconut plantation was further augmented by the Pakistan Agricultural

Research Council (PARC) through the import of 2,000 seed nuts of a dwarf variety in 1980 and 25,000 nuts of a tall variety from Sri Lanka in 1984.

The most important insect pests of the coconut are rhinoceros beetle (*Xyloryctes jamaicensis*), red palm weevil (*Rhynchophorus ferrugineus*), mealy bugs (*Nipaecoccus nipae*), scale insects and termites (*Reticulitermes* sp.).

Linseed

Linseed (*Linium ustitatissimum* L.) is called *Alsi* throughout Pakistan. It is an annual rabi (winter) crop grown for its seed and fiber. Its seed contains 20-25% protein and 30-40% oil, which has been increased to 45% in some cultivars. In Pakistan, the area under linseed has remained static (9,000-9,500 hectares) for the past 15 years. Punjab (47%) and Sindh (53%) are the major growing provinces (Hatam and Abbasi, 1994).

Linseed oil is used in the manufacture of paints and varnishes, oilcloth and linoleum. Its oilcake is used both as cattle feed and as manure. A good-quality fiber, used in the manufacture of canvas, cloth, and water-resistant pipes, can be extracted from the straw. The material remaining after the extraction of fiber can be pulped for use in the manufacture of paper and strawboard.

Generally, no serious insect pests have been observed on this crop. In rare cases the crop may be attacked by the capsule borer (*Heliothis* spp.), which can cause considerable loss in yield.

Safflower

Safflower (*Carthamus tinctorius* L.) is called *Kasumba, Pavari*, in Punjabi/Urdu, Sindhi, and Pashto, respectively (Chaudhry, 1986). Safflower is an annual, day-neutral rabi (winter) crop.

For many years, safflower cultivation has remained at 1.3-1.5 million hectares worldwide. In Pakistan, the cultivation of safflower was initiated rather late, probably during the early 1870's. The oil content of safflower seed is 32-36%. Because of the high content of unsaturated fatty acid (linoleic acid), it is used in the manufacture of soft margarines, as salad oil, and for other edible products. Its industrial uses include the manufacture of pharmaceuticals, paints and varnishes.

The main insect pests of safflower are capsule fly *(Canthiophilus helianthi*) and black aphid *(Uroleucon compositae*).

Soybean

Soybean is the most important oil and protein crop throughout the world. At present, soybean oil is the largest component of the world's edible oils and an ingredient of more than 50% of the world's high-protein meal. Since its introduction in Pakistan, the area under soybean has never exceeded 6000 hectares with some fluctuations during some growing years. In Pakistan, nearly 80% of the soybeans are grown in North West Frontier Province (NWFP), 19% in Punjab, and 1% in Sindh (Hatam and Abbasi, 1994). At present, the yield of soybean is quite low due to non-availability of a large number of commercial cultivars and grower preferences for other major crops. The selection and evaluation of short-duration cultivars is in progress which would make the cultivation of soybean possible and feasible in cotton and rice-based cropping systems in Punjab and Sindh provinces, and intercropping in orchards and sugarcane in North West Frontier Province (NWFP). Increase in soybean yield would help bridge the gap between edible oil production and consumption in addition to meeting the increasing demand for soybean meal from the poultry industry in the country.

Common insects infesting soybean are seed maggot, wireworm, white grub, thrips (*Thrips tabaci*), bean leaf beetle, armyworm (*Spodoptera litura*) and cutworm (*Agrotis*

ipsilon), hairy caterpillar, grasshopper, cabbage looper, earworm or bollworm, fall armyworm and stinkbug.

Integrated Pest Management in Pakistan

In Pakistan, the major reliance for the control of insect pests has been and still is on chemicals. The chemicals employed for insect pest management in oilseed crops is given in Table 1.

Table 1. Important insect pests of oilseed crops and chemicals used for their management (Amjad *et al.*, 2005a; Amjad *et al.*, 2005b; Hatam and Abbasi, 1994, Makhdoomi and Bajwa, 1975)

Insect Pests	Family	Order	Insecticides
Aphid (*Aphis gossypii*)	Aphididae	Hemiptera	Actara, Polo, Advantage
Armyworm (*Spodoptera litura*)	Noctuidae	Lepidoptera	Decis, Baythroid, Karate, Lorsban
Cabbage semilooper (*Thysanoplusia orichalcea*)	Noctuidae	Lepidoptera	Acephate, Bestox
Castor hairy caterpillar (*Euproctis lunata*)	Lymantriidae	Lepidoptera	Nuvacron, Lannate
Cutworm (*Agrotis ipsilon*)	Noctuidae	Lepidoptera	Lannate, Thiodan
Diamond back moth (*Plutella xylostella*)	Plutellidae	Lepidoptera	Decis, Baythroid, Lannate, Karate
Head borer (*Helicoverpa armigera*)	Noctuidae	Lepidoptera	Steward, Tracer, Lannate, Proclaim
Jassid (*Empoasca* sp.)	Cicadellidae	Homoptera	Actara, Polo, Advantage
Mustard sawfly (*Athalia proxima*)	Tenthridinidae	Hymenoptera	Curacron, Lorsban, Karate, Dipterex, Pay-Off
Painted bug (*Bagrada picta* Fab.)	Pentatomidae	Heteroptera	Thimet, Dimecron
Surface grasshopper (*Chrotogonus trachypterus*)	Acrididae	Orthoptera	Sevin
Whitefly (*Bemisia tabaci*)	Aleyrodidae	Hemiptera	Actara, Polo, Hostathion

In addition to chemical control, cultural control has also been in practice for some of the oilseed insect pests, which is highlighted in Table 2.

However, the popularity of cultural control is diminishing due primarily to the ease in the application of chemicals, availability of a large number of chemicals, and non-availability of cheaper labor (as large number of laborers are migrating to cities for alternative work).

Initial work on biological control in Pakistan was started in 1957. The potential for biological control has been more extensively studied and optimized on crops other than oilseeds. As regards to oilseeds, natural enemies (predators) of some of the insect pests are given in Table 3.

Table 2. Management of some important insect pests of oilseed crops through cultural methods (Amjad *et al.*, 2005a; Amjad *et al.*, 2005b; Baksh and Farooq, 1975)

Insect Pests	**Family**	**Order**	**Cultural Control**
Castor hairy caterpillar (*Euproctis lunata*)	Lymantriidae	Lepidoptera	Clean cultivation and removal of weeds
Dusky bug (*Nysius inconspicuous*)		Hemiptera	Eliminate the alternate hosts
Head borer (*Helicoverpa armigera*)	Noctuidae	Lepidoptera	Balanced fertilizers and pheromone traps
Mustard sawfly (*Athalia proxima*)	Tenthridinidae	Hymenoptera	Sowing at recommended time to avoid migration
Painted bug (*Bagrada picta*)	Pentatomidae	Heteroptera	Destruction of stubbles and dead plant material
Surface grasshopper (*Chrotogonus trachypterus*)	Acrididae	Orthoptera	Tillage operations to destroy eggs and eradicate weeds

Systematic work on IPM in Pakistan was started in 1980. The initiative was taken mainly by the private sector. Integrated Pest Management Consultants (IPMC) was one of the legends (Qureshi, 1999) with the major objectives to enhance agricultural productivity, cut crop production costs, reduce use of pesticides and conserve biodiversity through sustainable and environmentally friendly approaches for pest control utilizing natural renewable resources. IPMC has developed IPM technologies for mango, sugarcane and apple pests. Among the oilseed crops, emphasis has only been given to cotton, probably as a fiber crop and not as an oilseeds crop (Table 4).

In Pakistan, biological control has been attempted for some pests by introducing exotic natural enemies, redistribution of endemic natural enemies, and augmentation and conservation of local natural enemies. All these methods have been tried with varying degree of success.

Conclusions

Unfortunately, IPM on oilseed crops lags much behind that for other crops. Oilseed crops now need to be given due importance. There is still a significant gap between the production and consumption of oilseeds in Pakistan although substantial area is under oilseeds cultivation. This gap could be bridged with the same intensity of cultivation by the development of high yielding cultivars, improvement of soil fertility and effective management of insect pests. The latter should be achieved with minimum reliance on synthetic chemicals. Biological control based IPM has great promise for developing countries like Pakistan which cannot afford to spend large sums on extensive use of insecticides. As it is self-perpetuating, once a natural

enemy is established, little additional human involvement is required. Integrated Pest Management Consultants (IPMC) has collected a tremendous amount of information on the biology, ecology, and phenology of major insects and their natural enemies. With this available information, biological control of most of the important pests of the major crops can be attempted and utilized on a commercial basis. For this purpose serious collaborative efforts are needed with entomologists working closely with ecologists and environmental scientists.

Table 3. Important insect pests of oilseed crops and their possible biological control agents as explored in Pakistan (Amjad *et al.*, 2005a; Amjad *et al.*, 2005b)

Insect Pests	**Family**	**Order**	**Biological Control Agents**
Aphid (*Aphis gossypii*)	Aphididae	Hemiptera	*Chrysoperla carnea*, Coccinallid, *Chalcid*, *Ichneumonid*
Cabbage semilooper (*Thysanoplusia orichalcea*)	Noctuidae	Lepidoptera	Sparrows *Passer sp.*
Castor Hairy caterpillar (*Euproctis lunata*)	Lymantriidae	Lepidoptera	Braconid wasps
Diamond back moth (*Plutella xylostella*)	Plutellidae	Lepidoptera	*Diadegma insularis*, *Trichogramma* sp.
Head borer (*Helicoverpa armigera*)	Noctuidae	Lepidoptera	*Trichogramma, , Cotesia Bacillus thuringensis*, *Beauveria bassiana*
Jassid (*Empoasca* sp.)	Cicadellidae	Homoptera	*Chrysoperla carnea*, Coccinallid
Surface grasshopper (*Chrotogonus trachypterus*)	Acrididae	Orthoptera	*Anisodactylus* sp., *Epicauta* sp., *Asilus* sp.
Whitefly (*Bemisia tabaci*)	Aleyrodidae	Hemiptera	*Chrysoperla carnea*, Coccinallid

Table 4. Successes in biological control and IPM of cottonseed pests achieved in Pakistan (Qureshi, 1999)

Crop	**Insect Pests**	**Control measures**	**Remarks**
Cotton	Bollworms	Mass releases of *Trichogramma* sp.	Excellent control of bollworms
	Sucking pests	Conservation of predators	Excellent control of sucking pests

Bottlenecks

- Due importance has not been given and still is not being given to oilseed crops in Pakistan.

- Chemical pesticides are still quite cheap and easy to apply, therefore are the first choice for pest control by farmers.
- The enterprises dealing with chemicals are economically sound and have influence on our policies. Incentives are given by these companies to farmers, making their chemical products even more popular.
- There is a common expectation of Pakistani farmers for pest knock-down within a minimum time and with minimum effort. Additionally, they feel secure in achieving maximum yields by through the use of synthetic chemicals. This thinking is delaying the adoption of alternative strategies for pest management.
- There are yet no laws, at least under implementation, to penalize for pesticide residues in crop plants and plant products. Under such conditions, the farmers use pesticides injudiciously.
- Cultural methods have been common practice in Pakistan, but with the introduction of chemical means and with the massive movement of rural labor to urban areas, these methods have been diminished.
- Biological control needs a lot of money for initial establishment and optimization, which is big problem in developing countries like Pakistan.
- Biological control is difficult to apply and understand particularly by the farmers who are not well educated.
- The majority of Pakistani farmers have small holdings and are resource-poor. Hence they are reluctant to adopt IPM which is seen as risky. IPM can often only be successful if it is taken-on as a community. The systems to organize this co-operation is lacking.
- Implementation of IPM is rather a slow process and needs a lot of patience both from the scientists as well as from farmers. Like other strategies (chemical means) it does not match with “get the show on the road” mentality of our masses.
- To implement successful IPM, we will need to restructure and optimize our farming systems extensively, for which the farmers may not be willing.

With the growing concern about pesticides as a source of environmental pollution, as residues in food products, and pest-resistance issues, it is hoped that IPM will gain momentum in Pakistan leading towards clean, safe and prosperous agriculture in the future.

Acknowledgements

The authors are pleased to acknowledge Dr. Malgosia Jedryczka, for her help and guidance during the preparation of this manuscript.

References

Amjad, M., Haq, I. & Ali, N. 2005a: A key to insect pest management of sunflower crop. – Oilseed Research Program, National Agricultural Research Centre, Islamabad, Pakistan.

Amjad, M., Haq, I. & Ali, N. 2005b: A key to insect pest management of oilseed brassica. – Oilseed Research Program, National Agricultural Research Centre, Islamabad, Pakistan.

Anonymous. 1995: Oilseed Development Strategy. – National Oilseed Development Project, Ministry of Food Agriculture and Livestock, Government of Pakistan.

Anonymous. 2003: Annual Report 2002-2003. Oilseeds Research Programme. – Institute of Field and Horticultural Crops, National Agricultural Research Centre, Islamabad.

Anonymous. 2004: Agricultural Statistics of Pakistan 2003-04. – Ministry of Food Agricul-

ture and Livestock, Economic Wing, Islamabad, Pakistan.

Baksh, C.K. & Farooq, C.M. 1975: Insect pests of oilseed crops in Dera Ghazi Khan. – In: Proceedings of First National Oilseeds Seminar. Punjab Agricultural Research Institute, Lyallpur, 16-17 May, 1975. Agricultural Research Council, Islamabad, Pakistan. 132-138.

Chaudhry, A.H. 1986: Scope of Safflower in Pakistan. *In* Oilseed Research Development in Pakistan: A Perspective. – In: Proceedings of the National Seminar on Oilseed Research and Development in Pakistan. 7-9 May, 1985. Pakistan Agricultural Research Council, Islamabad, Pakistan. 114-130.

FAO. 2004: FAO, Year Book of Agricultural Statistics. – Food and Agriculture Organization of the United Nations. Rome, Italy.

Hatam, M. & Abbasi, G.Q. 1994: Oilseed Crops. – In: Crop Production. National Book Foundation. Islamabad, Nazir, S., Bashir, E. & Bantel, R. eds. 328-389.

Khan, A.H. & Sheikh, A.H. 1985: Sesame: An Important Oilseed Crop – *In Urdu*. Publication Division, Ayub Agricultural Research Institute, Faisalabad. 1-4.

Khan, A.H., Sheikh, A.H. & Ali, M. 1986: Cultivation of Castor – *In Urdu*. Publication Division, Ayub Agricultural Research Institute, Faisalabad. 1-6.

Makhdoomi, S.M.A. & Bajwa, M.H. 1975: Pests of oilseed crops and their control. – In: Proceedings of First National Oilseeds Seminar. Punjab Agricultural Research Institute, Lyallpur, 16-17 May, 1975: Agricultural Research Council, Islamabad, Pakistan. 119-124.

Qureshi, M.A. 1999: Integrated Pest Management in Pakistan. – In: Proceedings of the Workshop on Integrated Pest Management of Agricultural Pests for OIC Member Countries in Asia. 08-11 March, 1999. Integrated Pest Management Consultants and Eco-Conservation Consultants, Islamabad, Pakistan, Qureshi, M.A., Mohyuddin, A.I. & Irshad, M. eds. 162-174.

Shabbir, S.G. 1973. Cotton Pests, their distribution and seasonal prevalence. – Seminar on Insect Pests of Cotton. Pakistan Central Cotton Committee. 49-61.

Integrated oilseed rape protection in Belarus

Jadviha E. Piluk and Uladimir A. Radaunia
Institute of Arable Farming and Plant Breeding of the NAS of Belarus

Abstract: The main oilseed crop in Belarus is winter and spring rape and is cultivated on 80-140, 000 ha area or 2.5-3.0% of cultivated area. In total there are 1.2 million ha in Belarus fit for cultivation of winter rape and 2.8 million ha for spring rape. Thus, oilseed rape can cover 400-450 million ha in Belarus annually within a crop rotation.

The crop capacity of winter rape oilseeds in average in 2005 was 18 dt/ha, spring rape – 16 dt/ha. A lot of farms receive 25-35 dt/ha and even 40-50 dt/ha.

Depending on the overwintering survival of winter rape, the area sown to spring rape covers 45-80%. For the last 10 years in our republic, winter rape losses over winter reached 80-90% in 1994, 1999 and 2003. Around 30% of these losses were due to the break in crop technology cultivation (soil preparation, sowing terms, sowing norms and so on).

Oilseed rape in Belarus is affected each year with a complex of diseases, the most significant of which are the following:

Alternaria blight (*Alternaria*) which annually affects 20-80% of the crop, seed infection – 37-100%, sclerotinosis (*Sclerotinia sclerotiorum*), fusarial wilting of *F. oxysporum*, gray mould (*Botrytis cinerea*). On spring rape in sprouts period – wire stem (*Rhizoctonia, Pythium, Olpidium, Botrytis*), on winter rape in a period of hibernation – snow mould (*Fusarium nivale, Typhula incarnata, Sclerotinia trifoliorum*), bacteriosis (*Xanthomonas, Pseudomonas*), root rots, phomosis (*Phoma lingam*). Potentially the dangerous diseases are disease caused by Cylindrosporum (*Cylindrosporium concentricum*), blue mould (*Peronospora вrassicae*) and powdery mildew (*Erysipyhe cruciferarum*).

In recent years, between 95-98% of winter and spring rape varieties cultivated in Belarus are from the institute's breeding programme. All of them are tolerant to the most wide-spread diseases.

Five fungicides are permitted in Belarus, however they are very seldom used in production. For rape seeds dipping preparations WITAVAX 200, VINCIT are used favorably. Of great importance in disease control is a crop rotation and a cultivation technology directed towards good establishment.

The most harmful pest in oilseed rape cultivation in Belarus is the pollen beetle (*Meligethes aeneus*). Yield losses in the absence of insecticides are 20-35%. Spring rape in particular is affected by *M. aeneus* with yield losses reaching 35-60%. The cruciferous flea-beetles (*Phyllotera* spp.) are serious pests of establishment, particularly in spring rape crops. Increasing numbers of stem and seed seedpod weevils (*Ceutorhynchus pallidactylus*, *Ceutorhynchus assimilis*, respectively) have been noted. In some years, in winter rape sowings, the rape sawfly (*Athalia rosae*) has been observed. In dry years, the cabbage aphid (*Brevicoryne brassicae*) can be a serious pest. In Belarus, oilseed rape crops are usually treated 2-3 times during the growing season with insectides, especially KARATE, DECIS and FASTAK. Application of preparations are permitted in Belarus sowings. The most wide-spread of them are BUTISAN 400 (metazachlor), TROFI (acetochlor), TREFLAN (trifluralin), and TERIDOX (dimetachlor).

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 31-36

The occurrence of bees (Apoidea) on winter oilseed rape crops

Maria Kelm and Tomasz Strojny
Agricultural University of Wrocław, Plant Protection Department, ul. Cybulskiego 32; 50-205 Wrocław, Poland, KOR AR @ozi.ar.wroc.pl.

Abstract: The aim of this study conducted in 2004-2005 on six oilseed rape crops through the entire spring - summer vegetation period, was to assess the phenology and the distribution of Apoidea guilds in different landscapes. The analysis was based on visual counting of bees coupled with the line transect method. A total number of 7648 invidual bees were registered. The dominant species was *Apis mellifera* (63%). Among the wild bees, the most numerous group was Andrenidae (22%). The group Halictidae and *Bombus* spp. made up ca. 7% of the guild. The highest density of the honey bee was observed in transects running along neighbouring crops or along semi-natural plant assemblages, whereas the wild bees were recorded at their greatest numbers from transects close to field tracks, railway banks and drainage ditches. Wild species of bees appeared on oilseed rape crops in significant densities (32 % of the guild) before the flowering of the crop. At this early time, their recorded densities were the lowest in mid-field transects and at the edge-transects neighbouring semi-natural plant assemblages.

Key words: Winter oilseed rape, Apoidea, phenology, landscape

Introduction

Winter oilseed rape crops are the richest source of pollen and nectar for bees in agroecosystems. Honey productivity of rape flowers reaches about 170 kg/hectar. According to Williams (1986, 1987) rape plants sufficiently self- or cross-pollinated produce higher and better seed yield.

In Poland, 105 bee species, representatives of all European holarctic bee families, were recorded as pollinators on oilseed rape flowers (Banaszak, 1982). Most of these recorded species belong to three families: Andrenidae, Halictidae and Apidae. As they start activity when the average air temperature exceeds 8ºC, they are called early spring bees.

In our previous studies based on yellow water trap catches (Kelm, 2003; 2004) we demonstrated that bees occured on winter oiseed rape not only during the flowering stages of the crop, but that they also appeared in high numbers during the first half of April, i.e. at the beginning of spring vegetation period pre-flowering.

The aim of the present study was to determine the phenology and the habitat preferences of Apoidea in winter oilseed rape.

Materials and methods

Studies were conducted in 2004-2005 at Niciszów near Wrocław, Poland. Bees were recorded each year on 3 winter oilseed rape crops, every 2nd to 3rd day through the entire spring - summer vegetation period of the crop. To avoid the attraction of bees by the yellow water traps used in our first studies, we continued research using visual counting coupled with the line transects method. Each one of the transects was 1 m wide and 200 m long and they were all designed to be scanned during 20 minutes walking along them. Every year, bees were

observed on 3 winter oilseed rape fields of different areas and in different landscape contexts. Three transects, two of them along the opposite, longer field edges and one in the midle of the field, were marked on each one of the following fields:

2004
Field I – 0.66 ha, neighbouring a corn crop and a drainage ditch
Field II - 20 ha, neighbouring a field track and a meadow
Field III - 6 ha, neighbouring potato crops and a railway bank.

2005
Field I - 6 ha, neighbouring a small water course banked with willow (*Salix* spp.) and hawthorn (*Crategus* spp.) vegetation
Field II - 6 ha, neighbouring potato crops and a railway bank
Field III – 4.5 ha, neighbouring a corn crop and a field track.

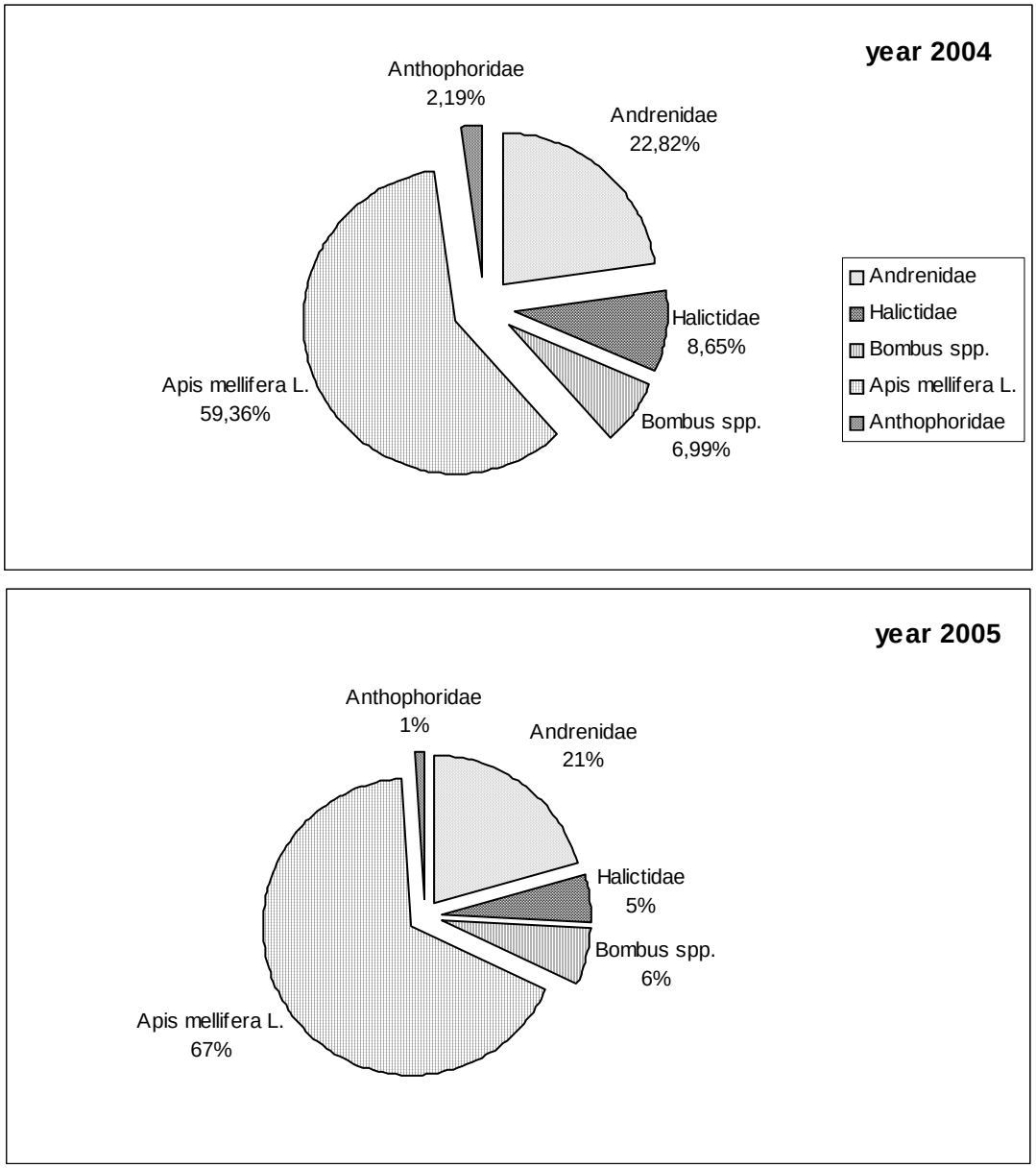


Fig. 1. Percentage structure of Apoidea occurrence in winter rape crops.

Results and discussion

The total number of bee individuals recorded was 7648. Percentage structure of the bee guild is shown in Figure. 1. In both years the honey bee, *Apis mellifera* L., dominated, making up 59 - 67% of the guild, probably due to the vicinity of apiaries, settled in the nearby village. The proportion among the wild bees was similar to that described in our first study (Kelm *et al.*, 2003). The most numerous groups were families Andrenidae (up to 23 %) and Halictidae (up to 9%). Anthophoridae, previously not recorded, occurred in much lower densities making up ca. 2% of the guild.

The genus *Bombus* was represented in the recorded material by the seven species, given here in order of their decreasing density: *B. terrestris* L., *B. lapidarius* L., *B. pascuorum* Scop., *B. lucorum* L., *B. pratorum* L., *B. ruderarius* Mull. and *B. hortorum* L. All species of *Bombus* made up 7% of the guild.

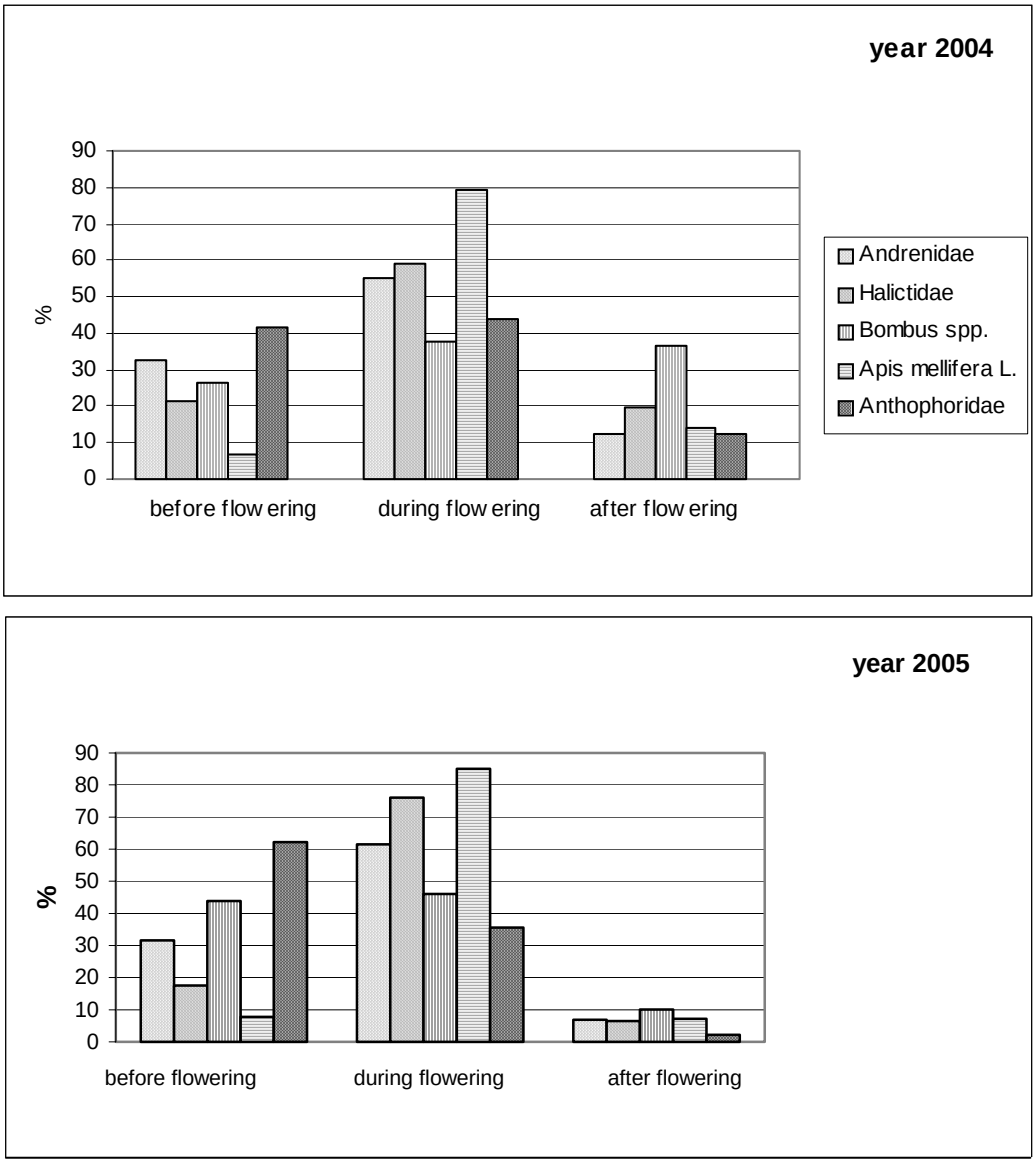


Fig. 2. Percentage number of Apoidea caught in water traps in winter rape at different growth stages.

The results confirm the data on the occurence of wild bees on the crop before flowering, obtained from yellow water traps (Figure. 2). Only the numbers of *A. mellifera*, did not exceed 10% its numbers observed through the entire vegetation period of the crop. The proportion of the wild bees of particular families in the overal guild density before the crop flowering was 20 - 40% in 2004 and 18 - 60% in 2005.

Of the total number of the honey bee individuals observed, 80% appeared on the crop during flowering. Bumblebees and Anthophoridae shared the smallest proportion of the guild at that time, compared to the rest of the vegetation period - ca. 40%. In both years, the highest densities of bumblebees were recorded after the crop flowering ceased.

Although the highest number of bees occured in the smallest field (0.66 ha), no correlation was demonstrated between crop area and bee density. In contrast, bee density rather seems to be dependent on the landscape structure.

Table 1 presents in greater detail the incidence of wild bees on the crop before flowering. Bumblebees, as well as Anthophoridae and Halictidae were scarce on transects neighbouring mid-field shrubs and meadows. Flowering willows and herbaceous spring vegetation presumably offer an abundance of food to these insects and they therefore choose not to search for it on the crop. The Andrenidae family was an exception, as the bees were recorded in significant density on such transects, particularly in 2005. Closer examination of the site revealed the presence of nests of these insects at the edge of the crop, in the vicinity of the mid-field shrubs. The discovery remains consistent with the calculated dispersion coefficient, having the highest value for Andrenidae, and indicating their patchy distribution at these crop edges which neighbour forest (Kelm *et al.*, 2003). In both years of the study, wild bees were hardly recorded before crop flowering from the mid-field transects. Only bumblebees and Andrenidae reached that "zone". On the contrary, transects neighbouring arable fields, still with no vegetation cover at that time, abounded with wild bees, as did transects set along the field tracks, the railway banks or along the drainage ditches. Among the bees, *Anthophoridae* occured mainly in the fields neibouring railway banks.

Table 1. Density of wild bees on particular types of transects before oilseed rape flowering.

	2004				2005			
Transects near to:	A*	H	ATH	B	A	H	ATH	B
mid-field shrubs, meadows	31	7	0	1	162	10	8	10
arable crops	107	27	16	26	47	12	6	32
field tracks, railway banks, drainage ditches	133	38	25	24	22	13	14	39
mid-field transects	27	2	0	22	8	0	0	19

* A- *Andrenidae,* H- *Halictidae,* ATH-*Anthophoridae,* B-*Bombus* spp.

Table 2 demonstrates the proportion beetwen honey bees and wild bees on the oilseed rape crop grown in different landscapes. This ratio was fairly similar on transects near to semi-natural plant assemblages and near to arable crops. On transects near to the field tracks, railway banks and drainage ditches, there were less honey bees and more wild bees, probably because such neighbouring areas offer more convenient habitats for wild bees` nests compared to the oilseed rape crop. On the mid-field transects, the honey bee was remarkably more abundant, which may be the result of the longer flight range of the species than that of the wild bees.

Table 2. The proportions of the honey bee and wild bees observed on different transects.

Transects near to:	Bees no. / transect	Honey bee no.	Honey bee %	Wild bees no.	Wild bees %
mid-field shrubs, meadows	706	451	64	254	36
arable crops	564	361	64	203	36
field tracks, railway banks, drainage ditches	565	314	55	251	45
mid-field transects	149	112	75	37	25

The results indicate that there is a need to improve conservation programs to protect these important pollinators. The insectcides recommended to control stem weevil and pollen beetle have treatment-timing guidelines (for application outside of flowering times) and information regarding the post-treatment safety interval for bees, in order to prevent bee poisonings. However, for the wild bees that are found on the crop before its flowering phase, the information about the product toxicity for bees seems more critical, as many insecticides having very short post-treatment safety intervals for bees are, at the same time, very toxic to them, for example the pyrethroids. Special attention should be paid to bumblebees; all species of which are legally protected. Their maximum abundance on winter oilseed rape is usually observed in April (Kelm *et al.,* 2004). The individuals in question are the overwintered, inseminated queens which fund new families and the insecticides applied at that time to the crop threatens both them and their progeny. The reduced use of chemicals, confined to the edge zone of 6.0 m width, starting from the field boundary, postulated recently by Klukowski (2004), does not make bee poisoning less likely, as their spatial distribution on the crop in early spring seems to overlap with the distribution of the pests.

In order to protect and enhance wild bee populations, trees and shrubs should be incorporated in the agricultural landscape. Many species of *Salix* and *Prunus* genera may provide bees with food resources in early spring (Ruszkowski, 1998). The insects might then postpone flying over the crops, where they are in a danger.

References

Banaszak, J. 1981: Metody określania liczebności pszczół na rzepaku ozimym. – Wiad. Entom. 10 (2): 113-119.

Banaszak, J. 1982: Występowanie i liczebność pszczół (*Hymenoptera, Apoidea)* na rzepaku ozimym. – Bad. Fizj. nad Polską Zach. 33 C: 117-127.

Kelm, M., Fostiak, I., Kaczmarzyk, M. & Klukowski, Z. 2003: Charakterystyka zgrupowania pszczół *Apoidea* na uprawach rzepaku ozimego. – Progress in Plant Protection 43(1): 172-181.

Kelm, M., Fostiak, I., Kaczmarzyk, M. & Klukowski, Z. 2004: Występowanie trzmieli (*Bombus spp*.) na rzepaku ozimym- implikacje dla ochrony upraw. – Rośliny Oleiste. 25: 187-194.

Klukowski, Z. 2004: Zmienność struktury przestrzennej populacji chowaczy łodygowych (*Ceutorhynchus pallidactylus* Marsh., *Ceutorhynchus napi* Gyll.) w uprawie rzepaku ozimego. – Zesz. Nauk. AR Wrocław, 478. Rozpr. 210: 122 pp.

Ruszkowski, A. 1998: Rośliny wskaźnikowe dla trzmieli (*Bombus* Latr.) krótkojęzyczkowych. – Pszczel. Zesz. Nauk. 42 (1): 321-325.

Williams, I.H., Martin, A.P. & White, R.P. 1986: The pollination reguirement of oil-seed rape (*Brassica napus* L.). – J. Agric. Sci. Camb. 106: 27-30.

Williams, I.H., Martin, A.P. & White, R.P. 1987: The effect of insect pollination on plant development and seed production in winter oilseed rape (*Brassica napus* L.). – J. Agric. Sci. Camb. 109: 353-357.

EU-Projects:
Current state of research

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 39-42

The EU project MASTER (MAnagement STrategies for European Rape pests): An update

Ingrid H. Williams
Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

MASTER (MAnagement STrategies for European Rape pests) is the acronym for the EU-funded project, entitled 'Integrated pest management strategies incorporating bio-control for European oilseed rape pests' (QLK5-CT-2001-01447). The project consortium has partners from six EU member states (Table 1).

The main aim of the project is to construct, develop, evaluate and promote an Integrated Pest Management (IPM) System for the European winter oilseed rape crop incorporating biological control of pests. The project is of four and a half years duration; it was initiated in December 2001 and will finish in May 2006. It is thus in its final year.

Table 1. Project partners and workpackages

Partner	**Lead scientist**	**Role**
Rothamsted Research, UK	Ingrid Williams	Coordinator
Georg-August University, Göttingen, Germany	Bernd Ulber	Leader, Workpackage: Parasitoids
BBA, Braunschweig, Germany	Wolfgang Büchs	Leader, Workpackage: Predators
University of Helsinki, Finland	Heikki Hokkanen & Ingeborg Menzler-Hokkanen	Leaders, Workpackages: Pathogens & Socio-economics
University of Agricultural Sciences, Sweden	Christer Nilsson	Leader, Workpackage: IPM Strategies
Agricultural University of Wroclaw, Poland	Zdzislaw Klukowski	Partner
University of Life Sciences, Estonia	Anne Luik	Partner

MASTER has five scientific objectives. These have been reported previously (Williams *et al*., 2002; 2003) and can be viewed on the project website

www.rothamsted.bbsrc.ac.uk/pie/master/master.htm

An extensive overview of progress during the first three years towards meeting these objectives was presented at the last meeting of the IOBC Working Group: Integrated Control in Oilseed Crops held at Rothamsted Research in 2004 (Williams *et al*., 2004). Since then, a further review of research progress focussing on the integration of results from the strategic workpackages on parasitoids, predators and pathogens into the more applied development of

an IPM Strategy for the European winter oilseed rape crop through 3 two-year collaborative field experiments in each of five partner countries has also been presented at an international meeting and published (Williams *et al*., 2005).

MASTER is an excellent example of a collaborative research team of internationally recognised oilseeds entomologists established and facilitated by the IOBC organisation. This collaboration arose through recognition, about ten years ago, by members of the IOBC Working Group: Integrated Control in Oilseed Crops of the need firstly for collation and exchange of information on naturally-occurring biological control agents of the pests of oilseed rape and secondly for collaborative experiments to integrate these agents into IPM Strategies for the crop. The former was achieved through the EU Framework 4 Concerted Action BORIS, the acronym for Biocontrol of Oilseed Rape Insects PestS, full title 'Minimising pesticide use and environmental impact by the development and promotion of bio-control strategies for oilseed rape pests' (FAIR-CT 96-1314) (Alford *et al*., 2000). Following completion of this Concerted Action, partners decided that the disparate literature on the naturally-occurring biological control agents they had collated should be made available to others and published it as a book (Alford, 2003), the first of its kind on this subject. This has proved to be a most valuable deliverable from this project and an essential database for the ongoing MASTER project.

The IOBC Working Group: Integrated Control in Oilseed Crops meetings have also provided MASTER project members with a valuable forum for dissemination of research results from the project. At the last meeting at Rothamsted Research, UK in 2004, representatives from six of the seven partner organisations attended and presented a total of 12 papers (see Bulletin IOBC/wprs vol. 27) reporting project results. At this 2005 meeting in Poznan, Poland, representatives of six of the seven partner organisations have again attended. Presentations include the latest research findings on key parasitoids of pests of winter and spring rape in Estonia, including the exciting finding of *Diospilus capito* as a key parasitoid on winter rape in Estonia (Veromann *et al*., this volume), demonstration of the use of upwind anemotaxis by *Tersilochus obscurator*, the key parasitoid of the cabbage stem weevil, during migration to the crop (Williams *et al*., this volume), the role of Staphylinid larvae as predators of pollen beetle larvae (Felsmann & Büchs, this volume) and the first results from the large-scale survey of oilseed rape growers in six EU Member States undertaken in 2003 (Menzler-Hokkanen *et al*. this volume).

Project results have also been disseminated widely at scientific and extension meetings in partner countries, at international conferences and in peer-reviewed journals; a full list of publications from the project has been placed on the project website. Work in the coming year will concentrate on the completion of data analyses for publication as papers, and for the Final Report and Technical Implementation Plan for the Commission. Technical Guidelines on the IPM Strategies and on conservation biocontrol of naturally-occurring parasitoids and predators for growers and advisors are being prepared.

The main venue for the dissemination of collated research findings from the whole project will be at the International Symposium 'Integrated Pest Management in Oilseed Rape', being organised by the British Crop Production Council (BCPC) on behalf of the MASTER project. It will be held in Göttingen, Germany, from 3-5 April 2006. Bernd Ulber is the Local Organiser. It will include sessions on parasitoids, predators, pathogens, IPM, the Phenological Models of key parasitoids for Decision Support, socio-economics and policy issues, and will include a review of the collated results from the collaborative field experiments. Invited and offered papers from other researchers working on integrated pest management of the crop will also be presented. The proceedings will be produced in CD format. Details of the Symposium can be found at:

www.rothamsted.bbsrc.ac.uk/pie/master/master.htm
and at
www.symposium-ipm-oilseed-rape.de

Acknowledgements

MASTER (QLK5-CT-2001-01447) is funded by the EU under its Framework 5 Quality of Life and Management of Living Resources programme. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK. I thank the following for their contributions to the project: Britt Ahman, Annely Kalle, Hassan Barari, Bertil Christensson, Suzanne Clark, Daniela Felsmann, Andrew Ferguson, Pierre Fernandez, Kirsa Fischer, Dave Frearson, Ross Holdgate, Andreas Johnen, Mariusz Kaczmarzyk, Reelika Kevvai, Beate Klander, Mart Kruus, Neil Mason, Helen Nuss, Ross Piper, Oliver Schlein, Emma Smith, Tiiu Tarang, Eve Veroman, Nigel Watts, Rainer Wedemeyer.

References

Alford, D.V. 2003 (ed): Biocontrol of oilseed rape insect pests. – Blackwell Science, Oxford, UK.

Alford, D.V., Ballanger, Y., Bűchi, R., Bűchs, W., Ekbom, B., Hansen, L.M., Hokkanen, H.M. T., Kromp, B., Nilsson, C., Ulber, B., Walters, K.F.A., Williams, I.H. & Young, J.E.B. 2000: Final Report EU Concerted Action CT96-1314 'BioControl of Rape Pests. – BORIS. 'Minimising pesticide use and environmental impact by the development and promotion of bio-control strategies for oilseed rape pests'.

Felsmann, D. & Büchs, W. 2006: Predation rates and assessment of Staphylinidae larvae from oilseed rape flower stands and their role in regulation of *Meligethes* larvae. – IOBC/wprs Bulletin 29(7): 161-163.

Menzler-Hokkanen, I., Hokkanen, H.M.T., Büchs, W., Klukowski, Z., Luik, A., Nilsson, C., Ulber, B. & Williams, I. 2006: Insect problems in European oilseed rape cultivation, and how to deal with them: the OSR Farmers' perspective. – IOBC/wprs Bulletin 29(7): 91-94.

Veromann, E. Kevväi, R & Luik, A. 2006: Oilseed rape pests and their parasitoids in Estonia. – IOBC/wprs Bulletin 29(7): Integrated Control in Oilseed Crops (this volume).

Williams, I.H., Barari, H. & Frearson, D. 2006: Upwind anemotaxis of the parasitoid *Tersilochus obscurator* during its migration flights to oilseed rape. – IOBC/wprs Bulletin 29(7): 165-172.

Williams, I.H., Büchs, W., Hokkanen, H., Menzler-Hokkanen, I., Johnen, A., Klukowski, Z., Luik, A., Nilsson, C. & Ulber, B. 2002: MASTER: Management Strategies for European Rape Pests - a new EU Project. – The BCPC Conference, Pests & Diseases, Brighton, 18-21 November 2002: 641-646.

Williams, I.H., Büchs, W., Hokkanen, H., Menzler-Hokkanen, I., Johnen, A., Klukowski, Z., Luik, A., Nilsson, C. & Ulber, B. 2003: Management Strategies for winter oilseed rape pests to enhance bio-control of rape pests. – Proceedings of the 11th International Rapeseed Congress, 6-10 July 2003, Copenhagen, Denmark: 1011-1013.

Williams, I.H., Büchs, W., Hokkanen, H., Klukowski, Z., Luik, A., Menzler-Hokkanen, I., Nilsson, C. & Ulber, B. 2004: The EU project MASTER (MAnagement STrategies for European Rape pests: a review of progress. –IOBC/wprs Bulletin 27(10): 3-16.

Williams, I.H., Büchs, W., Hokkanen, H., Menzler-Hokkanen, I., Johnen, A., Klukowski, Z., Luik, A., Nilsson, C. & Ulber, B. 2005: MASTER – Integrating biological control within

IPM for winter oilseed rape across Europe. – Proceedings of the BCPC International Congress, Crop Science & Technology, 31 October – 2 November, Glasgow, Scotland, UK: 301-308.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 43-48

SECURE – Stem Canker of oilseed rape: Molecular methods and mathematical modeling to deploy durable resistance (QLK5-CT-2002-01813)

Neal Evans[1], Bruce Fitt[1], Frank van den Bosch[1], Yong-Ju Huang[1], Stéphane Pietravalle[1], Maria Eckert[1], Konstantina Papastamati[1], Inez Demon[1], Zbigniew Karolewski[1,10], Thierry Rouxel[2]. Marie-Helene Balesdent[2], Isabelle Fudal[2], Lilian Gout[2], Hortense Brun[3], Didier Andrivon[3], Lydia Bousset[3], Peter Gladders[4], Xavier Pinochet[5], Annette Penaud[5], Malgorzata Jedryczka[6], Piotr Kachlicki[6], Anna Stachowiak[6], Julia Olechnowicz[6], Anna Podlesna[8], Ingrid Happstadius[7], Jan Meyer[7] and Michel Renard[9]
[1] Rothamsted Research, Harpenden, Herts., AL5 2JQ, U.K., e-mail: neal.evans@bbsrc.ac.uk, [2] INRA, Unité PMDV, Route de St Cyr, Versailles 78026, France, [3] INRA UMR BiO3P and [9] UMR APBV, Domaine de la Motte BP 35327, Le Rheu Cedex 35653, France, [4] ADAS Boxworth, Cambridge, CB3 8NN, UK, [5] CETIOM, B.P. no. 4, Thiverval-Grignon 78850, France, [6] IGR PAN, Strzeszynska 34, Poznan, 60-479, Poland, [7] Svalöf Weibull AB, Svalöv, SE-268 81, Sweden, [8] IUNG-Pulawy, Czartoryskich 8, 24-100 Pulawy, Poland,[10] Dept. of Phytopathology, August Cieszkowski Agricultural University, Dabrowskiego 159, 60-594 Poznan, Poland

Abstract: SECURE aims to deliver a model for deployment of cultivars with resistance to *Leptosphaeria maculans* (phoma stem canker/blackleg) to improve durability of resistance and minimise risk that the resistance will be overcome. The project has four main parts. Firstly, a life-cycle model for *L. maculans* has been developed and validated. Secondly, the fitness of virulent/avirulent isolates of the pathogen is being investigated; the results indicate a fitness penalty resulting from loss of the avirulence function for at least two resistance genes. Genomic analysis of avirulence and virulence loci of the pathogen is also being done. In addition, two *L.maculans* avirulent alleles (*AvrLm1* and *AvrLm6*) have been cloned. Thirdly, the effects of plant genetic background and environmental factors on durability of resistance are being analysed in both field experiments at a number of sites across Europe and controlled environment experiments. Lastly, models have been developed to investigate the effects of different schemes for deployment of resistance on durability of resistance so that recommendations can be made for a sustainable strategy for managing the disease. Results are being disseminated using a website (www.secure.rothamsted.ac.uk), scientific and popular publications and workshops.

Key words: Avirulence, *Brassica* napus, cloning, durability, *Leptosphaeria maculans*, *Phoma lingam*, resistance.

Introduction

Phoma stem canker (*Leptosphaeria maculans*) is the most damaging disease of oilseed rape in the UK (Fitt *et al*., 1997) and worldwide (West *et al.*, 2001; Fitt *et al.*, 2006). The mechanism of resistance to *L. maculans* operating in the leaf has been described as a gene-for-gene relationship, where the resistance or susceptibility of a cultivar depends on the presence of a major gene for resistance (*R*) in the host and a corresponding "effector" (*AVR*) gene in the pathogen. Specific recognition leads to the rapid onset of plant defence responses to fully protect the plant from the pathogen. There are currently nine *Brassica* spp. resistance genes

described, which correspond to nine effector genes in *L. maculans* (Delourme *et al*., 2004). However, when used in isolation, major gene resistance can be quickly overcome (Rouxel *et al*., 2003; Sprague *et al*., 2006). Quantitative adult plant resistance that is thought to be race non-specific and mediated by many genes also provides protection against *L. maculans* at the adult plant stage. For example, the cultivar "Jet Neuf", the best known source of quantitative resistance, was grown widely all over Europe during the 1970's and 1980's and is still very resistant to *L. maculans*. An aim of the SECURE project (QLK5-CT-2002-01813, EU Framework Programme 5) was to investigate whether durability of a major resistance gene (*Rlm6*) could be enhanced when the resistance was incorporated into a cultivar that also carried quantitative resistance (DarmorMX) in comparison to use in a susceptible background (Eurol MX).

Materials and methods

Workpackages

The SECURE project consists of five inter-related workpackages

- WP1 - Modelling the life cycle of *L. maculans*.
  A mechanistic model of the life cycle of *L. maculans* has been produced. This is being tested using existing data and new data from WP3.
- WP2 - Effects of pathogen variation at Avr loci on durability of resistance.
  Three avirulence loci (*AvrLm6*, *AvrLm1* and *AvrLm4*) are being analysed. The analysis includes cloning and functional characterisation of the gene. In addition, an analysis of the molecular events leading to virulence is being done. Fitness of virulent isolates has also been assessed in controlled environment and glasshouse experiments.
- WP3 - Effects of genotype/environment on durability of resistance.
  The influence of plant genetic background and of the environment on durability of resistance has been analysed through a series of field experiments across the main oilseed rape growing regions of Europe.
- WP4 - Strategy for sustainable deployment of durable resistance
  A model has been developed to investigate the interactions between resistance and avirulent/virulent *L. maculans* isolates. This has been used to develop new criteria to assess measures of durability of resistance (security, longevity, effectiveness) (van den Bosch & Gilligan, 2003) and to predict the effects of deployment strategy on durability of new resistance genes.
- WP5 - Diffusion of results
  In addition to management of the project, WP5 is associated with the delivery of results and recommendations to target groups/stakeholders. Results and recommendations are published on the SECURE website at www.secure.rothamsted.ac.uk.

Results and discussion

A prototype life-cycle model was developed during the first year of the SECURE project (WP1). Existing data and data generated during the first three years of the project have now been assimilated and model testing and validation has been done. Model fit to data was generally very good, with 50% of models fitted to datasets having an $R^2 < 80\%$. (e.g. Figure 1)

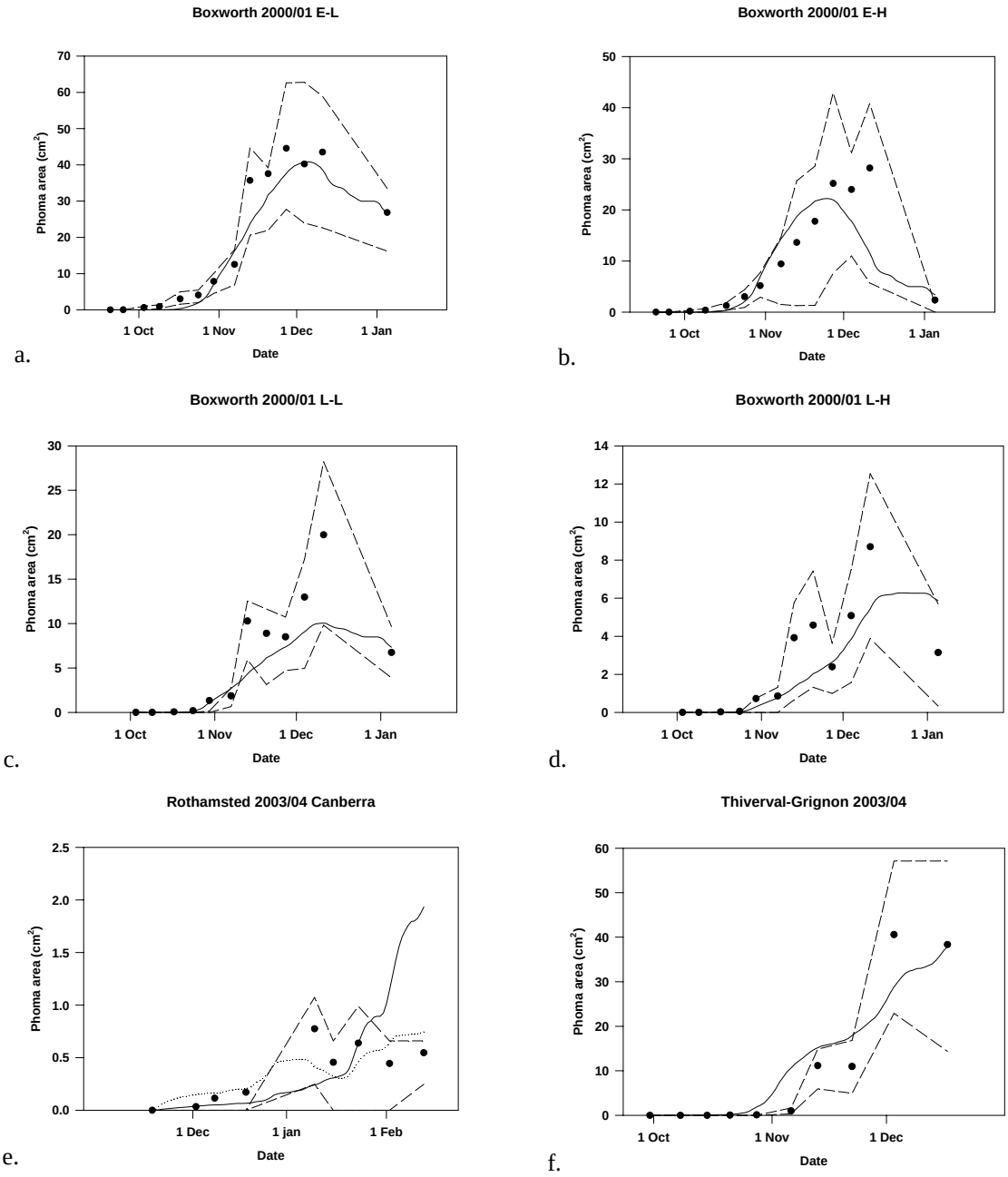


Fig. 1. Fit of stem canker disease progress models to field data from sites in the UK and France; a) Boxworth, UK, 2000/01 season, cultivar Pronto sown early (21 Aug 2000) at a low plant density, b) Boxworth, UK, 2000/01 season, cultivar Pronto sown early (21 Aug 2000) at a high plant density, c) Boxworth, UK, 2000/01 season, cultivar Pronto sown late (11 Sept 2000) at a low plant density, d) Boxworth, UK, 2000/01 season, cultivar Pronto sown late (11 Sept 2000) at a high plant density, e) Rothamsted, UK, 2003/04 season, cultivar Canberra sown 17 Sept 2003, f) Thiverval-Grignon, France, 2003/2004 season, cultivar Pinochet sown 5 Sept 2003. Points indicate disease score data, solid lines indicate the model predictions, dashed lines represent the 95% quartiles.

The field experimentation done during the SECURE project has demonstrated the dynamic nature of the interaction between the phoma stem canker pathogen *L. maculans*, the oilseed rape

host and the environment. The first two growing seasons (2002/03 and 2003/04) were characterised by a very dry autumn. Not only was the onset of the phoma leaf spot epidemic delayed in both seasons, but also poor emergence was a problem during the first season (2002/03), leading to a late epidemic on small plants or the loss of experiments. In contrast to the first two seasons, the 2004/05 season was typical, with moderate levels of phoma leaf spotting in the autumn and moderate to high severity of stem canker before harvest.

However, as in previous seasons, the MX (*Rlm6*) resistance in field experiments was not overcome (Figure 2) and the incidence and severity of phoma leaf spotting and stem canker were lower on plants with this resistance. From material tested, it appears that infections on these lines were probably caused by the related pathogen *L. biglobosa*. Indeed, some of the planned experiments on pathogenicity in WP3.2 could not be done due to the scarcity of pseudothecia of *L. maculans* on the MX material.

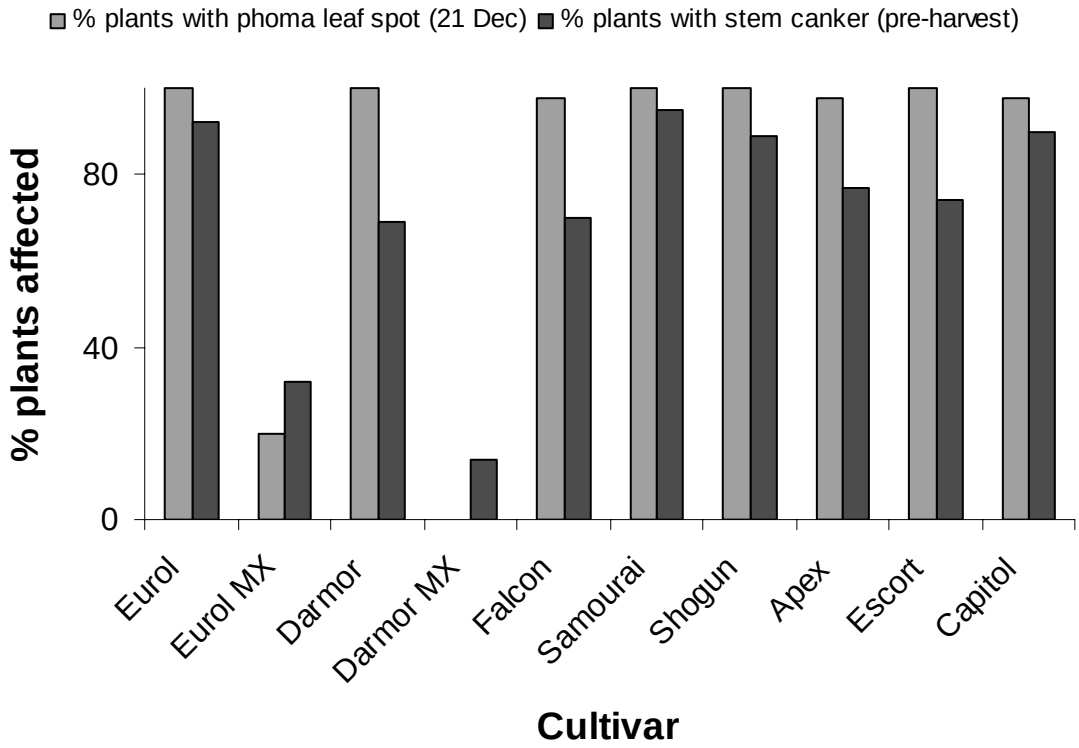


Fig. 2. Incidence of phoma leaf spot in December and of stem canker in June, at Boxworth, UK during the 2004/05 winter oilseed rape growing season.

Experiments done in WP2 using near-isogenic lines of the *L. maculans* pathogen to investigate pathogen fitness have produced interesting data in agreement with evidence from field experiments (Huang *et al*., 2006). The field data suggest a positive effect of the avirulence allele AvrLm4 on pathogenic fitness, and that the loss of this allele renders isolates less competitive than avirulent isolates under field conditions on cultivars without *Rlm4*. The highlight from the molecular work under WP2 is the cloning of *AvrLm1* (Gout *et al*., 2006) and *AvrLm6*. *L. maculans* is now one of the few fungal species for which two avirulence loci have been cloned. Research now focuses on understanding the function of *AvrLm6* and analysis of sequences of virulent isolates to understand molecular evolution towards virulence. In addition, data should become available from the *L. maculans* genome sequence project (http://www.genoscope.cns.fr/) during 2006/07, which should greatly help understanding of the molecular genetics of *L. maculans*.

Other important research done during the SECURE project has been the transformation of *L. maculans* with GFP and/or DsRed (Eckert *et al*., 2005). These transformed isolates have been used to follow growth of the fungus in *B. napus* near isogenic lines (NIL) with or without MX (*Rlm6*) resistance under different temperature and wetness conditions (Huang *et al*., 2006). The results from this work have greatly enhanced our knowledge of the infection process and the rate and extent of *in planta* growth on different cultivars.

The results from work done to model durability of resistance have provided some interesting conclusions. This work will be extended to a series of recommendations to policy makers and the breeding industry as to the best way to "manage" resistance genes to maintain durability of resistance (Pietravalle *et al*., 2006). The results from field experimentation that compares durability of resistance conferred by the major resistance gene *Rlm6* alone in a susceptible background (EurolMX) or in a resistant background (DarmorMX) under recurrent selection over 4 years (WP3) should confirm results from the modelling work. The experiments were designed to assess the usefulness of combining different types of resistance (major gene/quantitative) in breeding programmes. For example, previous recurrent selection experiments indicated that resistance was overcome in 3-4 seasons (Brun *et al*, 2000; Delourme *et al*., 2006).

A major priority of the project is knowledge transfer of results and recommendations to target audiences such as plant breeding companies and extension services. For example, one of the SECURE research partners CETIOM (Centre Technique Interprofessionel des Oléagineux Métropolitains) has developed a "diversification scheme" that encourages French growers to make an informed choice about the cultivars that are grown within the rotation based on the resistance genes carried by the individual cultivars (Gladders *et al*., 2006). Schemes such as these, in association with survey data that provides analysis of the population structure of *L. maculans* both on a national level (i.e. France; Balesdent *et al*., 2006) and an international level (i.e. Europe; Stachowiak *et al*., 2006), provide opportunities for the industry to manage available resistance more effectively.

Acknowledgement

The authors gratefully acknowledge funding from the European Commission under the Framework 5, Quality of Life Programme (QLK5-CT-2002-01813). Rothamsted Research also receives funding from the UK Department for Environment, Food and Rural Affairs and the Biotechnology and Biological Sciences Research Council.

References

Balesdent, M.-H., Louvard, K., Pinochet, X. & Rouxel, T. 2006. A large-scale survey of races of *Leptosphaeria maculans* occurring on oilseed rape in France. – European Journal of Plant Pathology 114: 53-65

Brun, H., Levivier, S., Somda, I., Ruer, D., Renard, M. & Chèvre, A.M. 2000. A field method for evaluating the potential durability of new resistance sources: Application to the *Leptosphaeria maculans Brassica napus* pathosystem. – Phytopathology 90: 961-6.

Delourme, R., Pilet-Nayel, M.L., Archipiano, M., Horvais, R., Tanguy, X., Rouxel, T., Brun, H., Renard, M. & Balesdent, M.H. 2004. A cluster of major specific resistance genes to *Leptosphaeria maculans* in *Brassica napus*. – Phytopathology 94: 578-583.

Delourme, R., Chèvre, A.M., Brun, H., Rouxel, T., Balesdent, M.H., Dias, J.S., Salisbury, P.& Rimmer, S.R. 2006. Major gene and polygenic resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus*). – European Journal of Plant Pathology 114: 41-52.

Eckert, M., Maguire, K., Urban, M., Foster, S., Fitt, B., Lucas, J. & Hammond-Kosack, K. 2005. *Agrobacterium tumefaciens*-mediated transformation of *Leptosphaeria spp*. and *Oculi-macula* spp. with the reef coral gene DsRed and the jellyfish gene gfp. – FEMS Microbiology Letters 253: 67-74.

Fitt, B.D.L, Gladders, P., Turner, J.A., Sutherland, K.G., Welham, S.J. & Davies, J.M.L. 1997. Prospects for developing a forecasting scheme to optimise use of fungicides for disease control on winter oilseed rape in the UK. – Aspects of Applied Biology 48 (Optimising Pesticide Applications): 135-142.

Fitt, B.D.L., Brun, H., Barbetti, M.J. & Rimmer, S.R. 2006. World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed Rape (*Brassica napus*). – European Journal of Plant Pathology 114: 3-15.

Gladders, P., Evans, N., Marcroft, S. & Pinochet, X. 2006. Dissemination of information about management strategies and changes in farming practices for the exploitation of resistance to *Leptosphaeria maculans* (phoma stem canker) in oilseed rape cultivars. – European Journal of Plant Pathology 114: 117-126.

Gout, L., Fudal, I., Kuhn, M.-L., Blaise, F., Eckert, M., Cattolico, L., Balesdent, M.-H., Rouxel, T. 2006. Lost in the middle of nowhere: The AvrLm1 avirulence gene of the Dothideomycete *Leptosphaeria maculans*. – Molecular Microbiology 60: 67-80.

Huang, Y.-J., Evans, N., Li, Z.-Q., Eckert, M., Chèvre, A.-M., Renard, M. & Fitt, B.D.L. 2006. Temperature and leaf wetness duration affect phenotypic expression of Rlm6-mediated resistance to *Leptosphaeria maculans* in *Brassica napus*. – New Phytologist 170: 129-141.

Huang, Y.-J., Li, Z.-Q., Evans, N., Rouxel, T., Fitt, B.D.L. & Balesdent, M.-H. Fitness cost associated with loss of the AvrLm4 avirulence function in *Leptosphaeria maculans* (phoma stem canker of oilseed rape). – European Journal of Plant Pathology 114: 77-89.

Pietravalle, S., Lemarié, S. & van den Bosch, F. 2006. Durability of resistance and cost of virulence. – European Journal of Plant Pathology 114: 107-116.

Rouxel, T., Penaud, A., Pinochet, X., Brun, H., Gout, L., Delourme, R., Schmit, J. & Balesdent, M.-H. 2001. A 10-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the *Rlm1* resistance gene of oilseed rape. – European Journal of Plant Pathology 109: 871-881.

Sprague, S.J., Balesdent, M.-H., Brun, H., Hayden, H.L., Marcroft, S.J., Pinochet, X., Rouxel, T. & Howlett, B.J. 2006. Major gene resistance in *Brassica napus* (oilseed rape) is overcome by changes in virulence of populations of *Leptosphaeria maculans* in France and Australia. – European Journal of Plant Pathology 114: 33-40.

Stachowiak, A., Olechnowicz, J., Jedryczka, M., Rouxel, T., Balesdent, M.-H., Happstadius, I., Gladders, P., Latunde-Dada, A.O. & Evans, N. 2006. Frequency of avirulence alleles in field populations of *Leptosphaeria maculans* in Europe. – European Journal of Plant Pathology 114: 67-75.

van den Bosch, F. & Gilligan, C.A. 2003. Measures of durability of resistance. – Phytopathology 93: 616-625.

West J.S., Kharbanda P.D., Barbetti M.J., Fitt B.D.L. 2001: Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. – Plant Pathology 50: 10-27.

Effects of production measures on plant health

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 51-57

Control of oilseed rape pests during flowering and pod development with combined application of insecticides and fungicides in 2003 – 2005

Gustaw Seta[1] and Marek Mrówczyński[2]
[1]*Institute of Plant Protection, Sośnicowice Branch, ul. Gliwicka, 44-153 Sośnicowice, Poland*
[2]*Institute of Plant Protection, ul. Miczurina 20, 60-318 Poznań, Poland*

Abstract: In 2003 – 2004, at the Sośnicowice Branch of the Institute of Plant Protection, Poznań, investigations were made of tank-mix applications of modern production technologies of winter oilseed rape. This paper reports results from synchronous application of insecticides to control cabbage seed weevil (*Ceutorhynchus assimilis* Payk) and brassica pod midge (*Dasyneura brassicae* Winn.) and fungicides to control fungal pathogens at the flowering stages of the crop.

In 2003, one trial was conducted with two insecticides – Karate Zeon 050 CS (lambda-cyhalothrin) and Trebon 10 SC (ethofenprox). These insecticides were used individually or mixed with fungicides - Alert 375 SC (flusilazole + carbendazim), Horizon 250 EW (tebuconazole) and Amistar 250 SC (azoxystrobin). In 2004, three trials were conducted in which the same insecticides were used together with Proteus 110 OD (thiachloprid, deltamethrin), Calipso 480 SC (thiachloprid), and Trebon 10 SC (ethofenprox), and the same fungicides, together with Alert 375 SC and Amistar 250 SC. These three trials differed only in date and amount of protective treatments. In 2005, as in 2004, three trials were conducted with three insecticides - Calipso 480 SC, Patriot 100 EC (deltamethrin), Proteus 110 OD, and two fungicides - Alert 374 SC and Horizon 250 EW.

The most effective control of cabbage seed weevil and brassica pod midge was obtained in the trials which used two protective treatments in a one-week period. The highest increase in yield was obtained in the investigations with the treatments applied at BBCH growth stage 65 (trial 1) or at both growth stage 65 and 67 (trial 2).

Key words: Oilseed rape, control, cabbage seed weevil, brassica pod midge, insecticides, fungicides, tank-mix application

Introduction

An important task in oilseed rape crop production is the control of the several pests that attack this plant during its development. In Poland, crop losses due to pest damage can, in extreme cases, reach 80 % (Mrówczyński *at al*., 1993; Muśnicki *at al*., 1994).

In Southern Poland, where the investigation reported here was conducted, cabbage seed weevil (*Ceutorhynchus assimilis* Payk.) and brassica pod midge (*Dasyneura brassicae* Winn.), besides cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsh.) and pollen beetle (*Meligethes aeneus* F.), are the most numerous and damaging pests of winter oilseed rape (Czajkowska, 1978; Szulc, 1996; Seta, 2003).

The cabbage seed weevil adults appear in the winter oilseed rape crop at the start of flowering. This co-incides with the appearance of the brassica pod midge. Brassica pod midge oviposition and larval development depends mostly upon the cabbage seed weevil density. Though the brassica pod midge is able to lay eggs into young, uninjured pods, in 88 % of cases it uses pods damaged by cabbage seed weevil oviposition (Skrocki, 1979; 1989).

Yield loss caused by these two pests, increased linearly by 1.7% for each 1% increase in the percentage of infested pods when larval infestation of pods exceeded 23% (Bountin, 1999). Migration of the cabbage seed weevil to winter oilseed rape crops occurs simultaneously with the migration of the brassica pod midge. Therefore, the control of both pests should be carried out at the same time, just before full flowering when the first pods are being formed (Skrocki Cz., 1979).

At this same time, some fungal diseases: *Botrytis cinerea, Alternaria* ssp., can also infect the oilseed rape plants. Therefore, investigations with tank-mix applications of insecticides and fungicides for control of winter oilseed rape pests and diseases at the flowering period were conducted.

Materials and methods

The investigations were conducted on winter oilseed rape cv. Silvia in 2003, cv. Californium in 2004 and cv. Carousel in 2005.

In 2004 one experiment was conducted in which the crop was sprayed at the full flowering growth stage – BBCH 65. Two insecticides were sprayed individually or mixed with three fungicides. Table 1 lists the insecticides and fungicides tested and their doses per ha.

In 2004 and 2005, three trials were conducted. In trial number 1, just as in 2003, the crop was treated only once at the full flowering growth stage (BBCH 65). In trial number 2, the crop was treated twice: the first treatment was applied at the full flowering growth stage (BBCH 65) and the second treatment was applied one week later, at growth stage BBCH 67, when the most of the flower petals had dropped. Trial number 3, like trial number 1, was treated only once but like trial number 2, the treatment was applied at growth stage BBCH 67. Tables 2 and 3 list the insecticides and fungicides tested and their doses per ha.

Cabbage seed weevil adult infestation was assessed on plants over 1 m2 along the plot diagonal before the application and one day afterwards. Effectiveness of insecticides and their mixtures with insecticides were counted by using Henderson-Tilton formula (Ciba-Geigy AG, 1981):

$$\% \text{ effectiveness} = \left(1 - \frac{Ta}{Tb} \times \frac{Cb}{Ca}\right) \times 100$$ where:

Tb - mean insect infestation before spraying of treated plot,
Ta - mean insect infestation after spraying of treated plot,
Cb - mean insect infestation before spraying of untreated plot (control),
Ca - mean insect infestation after spraying of untreated plot (control).

Plants were examined for possible phytotoxic effects of the mixtures 3, 7 and 14 days after treatment. Estimates of the effectiveness of separate chemicals were made 21 days after applications, before the pods started to split. Fifteen primary racemes were collected from each plot along a diagonal transect, and then they were assessed for the degree of infestation by the cabbage seed weevil and brassica pod midge. The percentage of injured pods were calculated.

After harvesting the crops, the seed yields were recorded. The increased yield (t/hectare) relative to untreated controls of the tank-mix application of insecticides and fungicides were calculated.

All combinations of tank-mixed pesticides were subjected to physical tests and the stability of water suspension and emulsion were evaluated.

Results and discussion

Tables 1 – 4 present the data from the 2003 – 2005 trials and show the effects of timing and dose rate of mixed-tank applications of insecticides and fungicides on pest incidence, pod damage and yields of oilseed rape. No phytotoxic effects were seen in any of the trials. All of the insecticides and their mixtures with fungicides were sufficiently effective in controlling pod pests in flowering winter oilseed rape.

Tank-mix application of insecticides and fungicides during flowering increased the effectiveness of brassica pod midge and cabbage seed weevil control in many cases. All insecticides and their mixtures with fungicides increased yields – the differences were almost always statistically significantly different from the control.

Application of different compounds in one spraying operation permits the reduction of production costs of winter rape. However, prophylactic application of insecticides should be reconsidered and attempts made to practice judicial use and integration with more biorational approaches.

References

Buntin, G.D. 1999: Damage loss assessment and control of cabbage seedpod weevil (Coleoptera: Curculionidae) in winter canola using insecticides. – Journal of Economic Entomology 92: 220-227.

Ciba-Geigy AG, Bazylea, Szwjcaria 1981: Podręcznik doświadczalnictwa polowego w ochronie roślin. Werner Puntener, Agro-Division, Ciba Geigy AG. – Ocenianie doświadczenia: 39-41.

Czajkowska, M. 1978: Badania nad pryszczarkiem kapustnikiem (*Dasyneura brassicae* Winn.). III. Metoda oceny stopnia porażenia roślin. – Rocz. Nauk Rol., Ochrona Roślin. 17(2): 169-170.

Mrówczyński, M, Ciesielski, F. & Witkowski, W. 1987: Application of new insecticides in the control of *Ceutorhynchus assimilis* and *Dasyneura brassicae* in winter rape. – Proc. 7 Intern. Rapeseed Congr.: 1170-1176.

Mrówczyński, M., Widerski, K., Przyłęcka, E., Paradowski, A., Pałosz, T., Wałkowski, T. & Heiman, S. 1993: Ochrona roślin w integrowanych systemach produkcji rolniczej. – Rzepak ozimy, Instrukcja upowszechnieniowa: 45-58.

Muśnicki, Cz., Toboła, P. & Mrówczyński, M. 1994: Produkcyjne skutki zaniechania ochrony rzepaku przed szkodnikami. – Materiały 34 Sesji Nauk. Inst. Ochr. Roślin (2): 17-21.

Skrocki, Cz. 1979: Zależność składania jaj pryszczarka kapustnika (*Dasyneura brassicae* Winn.) od występowania chowacza podobnika (*Ceutorhynchus assimilis* Payk.) na rzepaku ozimym. – Rocz. Nauk Rol. seria E 9(2): 149-157.

Skrocki, Cz. 1979: Najważniejsze zagadnienia z zakresu ekologii pryszczarka kapustnika (*Dasyneura brassicae* Winn.). – Rocz. Nauk Rol. 1, seria E 9 (1): 209-217.

Szulc, P. 1996: Uwagi na temat ochrony rzepaku przed szkodnikami. – Postępy Nauk Rolniczych, nr 2: 87-96.

Seta, G. 2003. Łączne stosowanie insektycydów i fungicydów w ochronie rzepaku ozimego w okresie kwitnienia i zawiązywania łuszczyn. –Rośliny Oleiste 24(1): 183-191.

Table 1. The effectiviveness of combined application of insecticides and fungicides on yield, adult cabbage seed weevil (*Ceutorhynchus assimilis* Payk.) incidence and pod damage caused by *C. assimilis* and brassica pod midge (*Dasyneura brassicae* Winn.) in crops of winter oilseed rape in 2003.

Treatments	Dose (kg/ha)	*C. assimilis* incidence 1 day after spraying		Damaged pods caused by *C. assimilis* and *D. brassicae*		Seed yields	
		Mean no/m^2	Effective-ness%	%	Effective-ness(%)	Yield (t/ha)	Increased yield (t/ha)
CONTROL	-	14.00 **b**	-	24.25 **e**	-	2.29 **a**	-
Karate Zeon 050 CS	0.15	01.25 **a**	85.18	10.00 **a-d**	58.76	3.31 **bc**	1.02
Karate Zeon 050 CS + Alert 375 SC	0.15 + 1.20	01.75 **a**	81.94	08.00 **a**	67.01	3.24 **b**	0.95
Karate Zeon 050 CS + Horizon 250 EW	0.15 + 1.20	02.75 **a**	59.68	08.25 **ab**	65.98	3.57 **bc**	1.27
Karate Zeon 050 CS + Amistar 250 SC	0.15 + 1.00	04.50 **a**	56.02	09.00 **ab**	62.89	3.46 **bc**	1.17
Trebon 10 SC	0.50	04.00 **a**	48.17	14.50 **b-d**	40.21	3.17 **b**	0.89
Trebon 10 SC + Alert 375 SC	0.50 + 1.20	01.75 **a**	73.65	15.50 **cd**	36.08	3.25 **b**	0.96
Trebon 10 SC + Horizon 250 EW	0.50 + 1.20	02.25 **a**	69.43	09.25 **a-c**	61.86	3.67 **c**	1.38
Trebon 10 SC + Amistar 250 SC	0.50 + 1.00	00.75 **a**	93.04	15.75 **d**	35.05	3.48 **bc**	1.19
LSD 0.05		04.16		06.50		0.41	

Table 2. The effectiviveness (Ef.) of combined application of insecticide and fungicides on adult cabbage seed weevil (*Ceutorhynchus assimilis* Payk.) incidence and pod damage by *C. assimilis* and brassica pod midge (*Dasyneura brassicae* Winn.) in winter oil seed rape in 2004.

Treatments	Dose kg/ha	**Trial 1:** Application at BBCH 65				**Trial 2:** Applications at BBCH 65 and 67				**Trial 3.** Application at BBCH 67			
		C. assimilis		Damaged pods		*C. assimilis*		Damaged pods		*C. assimilis*		Damaged pods	
		Mean no/m^2	*Ef.* %	%	*Ef.* %	Mean no/m^2	*Ef.* %	%	*Ef.* %	Mean no/m^2	*Ef.* %	%	*Ef.* %
CONTROL	-	16.50 **c**	-	10,35 **c**	-	7.00 **b**	-	24.93 **c**	-	13.75 **b**	-	28.12 **c**	-
Proteus 110 OD	0.60	09.25 **b**	62.93	04.65 **ab**	70.29	0.00 **a**	10.00	02.52 **a**	92.72	00.25 **a**	98.27	04.08 **a**	86.24
Proteus 110 OD + Alert 375 SC	0.60+ 1.20	05.00 **d**	76.99	02.80 **ab**	79.46	0.50 **a**	86.95	01.43 **a**	95.64	00.25 **a**	98.26	04.85 **a**	83.45
Proteus 110 OD + Amistar 250 SC	0.60+ 1.20	05.75 **a**	71.14	03.50 **ab**	71.99	0.75 **a**	81.68	02.17 **a**	92.49	00.50 **a**	96.71	08.78 **a**	71.77
Calipso 480 SC	0.10	04.75 **a**	76.16	03.17 **ab**	74.59	0.75 **a**	81.68	03.90 **a**	86.84	00.75 **a**	94.55	06.70 **a**	76.18
Calipso 480 SC + Alert 375 SC	0.10+ 1.20	06.25 **a**	63.02	02.38 **ab**	77.60	0.50 **a**	90.04	03.55 **a**	85.95	00.50 **a**	96.94	09.00 **a**	73.10
Calipso 480 SC + Amistar 250 SC	0.10+ 1.20	04.25 **a**	81.63	06.97 **bc**	51.95	0.75 **a**	77.29	04.90 **a**	81.59	00.75 **a**	94.60	07.58 **a**	73.35
Trebon 10 SC + Amistar 250 SC	0.50+ 1.00	05.75 **a**	58.59	06.80 **a-c**	21.92	0.25 **a**	93.24	10.53 **b**	59.42	00.75 **a**	94.31	16.20 **b**	39.87
LSD 0.05		02.91		04.46		1.75		04.54		04.43		06.48	

Table 3. The effectiviveness (Ef.) of combined application of insecticide and fungicides on cabbage seed weevil (*Ceutorhynchus assimilis* Payk.) adult incidence and pod damage by *C.assimilis* and brassica pod midge (*Dasyneura brassicae* Winn in winter oilseed rape in 2005.

Treatments	Dose kg/ha	**Trial 1**: Application at BBCH 65				**Trial 2:** Applications at BBCH 65 and 67				**Trial 3.** Application at BBCH 67			
		C. assimilis		Damaged pods		*C. assimilis*		Damaged pods		*C. assimilis*		Damaged pods	
		Mean no/m^2	*Ef.* %	%	*Ef.* %	Mean no./ m^2	*Ef.* %	%	*Ef.* %	Mean no./ m^2	*Ef.* %	%	*Ef.* %
CONTROL	-	0.90 **b**	-	20.80 **b**	-	1.02 **b**	-	23.48 **c**	-	There were no adult insects in the trials		21.15 **e**	-
Calipso 480 SC	0.10	0.06 **a**	92.06	11.97 **a**	42.43	0.04 **a**	96.57	06.08 **b**	74.12			16.68 **de**	21.16
Calipso 480 SC + Alert 375 SC	0.10 + 1.20	0.09 **a**	89.23	09.75 **a**	53.13	0.08 **a**	90.02	06.55 **b**	72.10			11.20 **a-c**	47.04
Calipso 480 SC + Horizon 250 EW	0.10 + 1.20	0.08 **a**	88.51	08.68 **a**	58.29	0.04 **a**	96.46	04.20 **ab**	82.11			12.72 **b-d**	39.83
Patriot 100 EC	0.075	0.03 **a**	94.97	13.28 **a**	36.18	0.02 **a**	98.11	07.55 **b**	67.84			06.88 **a**	67.49
Patriot 100 EC + Alert 375 SC	0.075 + 1.20	0.04 **a**	95.43	11.97 **a**	42.43	0.07 **a**	93.14	07.28 **b**	69.01			14.97 **cd**	29.20
Patriot 100 EC + Horizon 250 EW	0.075 + 1.20	0.02 **a**	97.21	07.62 **a**	63.34	0.02 **a**	98.17	05.75 **b**	75.51			16.80 **de**	20.57
Proteus 110 OD	0.60	0.02 **a**	96.53	09.38 **a**	54.93	0.07 **a**	94.51	01.30 **a**	94.46			12.22 **b-d**	42.20
Proteus 110 OD + Alert 375 SC	0.60 + 1.20	0.07 **a**	92.35	07.47 **a**	64.06	0.10 **a**	90.20	01.80 **a**	92.33			08.75 **ab**	58.63
Proteus 110 OD + Horizon 250 EW	0.60 + 1.20	0.09 **a**	86.69	08.70 **a**	58.17	0.05 **a**	93.46	01.07 **a**	95.42			10.93 **a-c**	48.35
LSD 0.05		0.09		05.93		0.11		03.76				04.70	

Table 4. The effect of combined application of insecticide and fungicides on yield in winter oilseed rape in 2004 - 2005.

Treatments	Dose (kg/ha)	Trials and terms of applications					
		Trial 1: Application at BBCH 65		**Trial 2:** Application at BBCH 65 and 67		**Trial 3:** Application at BBCH 67	
		Yield (t/ha)	*Increased yield (t/ha)*	*Yield (t/ha)*	*Increased yield (t/ha)*	*Yield (t/ha)*	*Increased yield (t/ha)*
2004							
CONTROL	-	3.82 **a**	-	3.79 **a**	-	4.52 **a**	-
Proteus 110 OD	0.60	5.71 **c**	1.89	5.33 **b**	1.54	5.38 **b**	0.86
Proteus 110 OD + Alert 375 SC	0.60 + 1.20	5.34 **c**	1.53	5.04 **b**	1.26	5.51 **bc**	1.00
Proteus 110 OD + Amistar 250 SC	0.60 + 1.20	5.47 **c**	1.65	5.91 **c**	2.12	5.81 **c**	1.29
Calipso 480 SC	0.10	5.37 **c**	1.55	5.42 **b**	1.64	5.29 **b**	0.77
Calipso 480 SC + Alert 375 SC	0.10 + 1.20	5.37 **c**	1.55	5.44 **bc**	1.65	5.36 **bc**	0.84
Calipso 480 SC + Amistar 250 SC	0.10 + 1.20	5.38 **c**	1.57	5.56 **bc**	1.77	5.46 **bc**	0.95
Trebon 10 SC + Amistar 250 SC	0.50	4.58 **b**	0.77	5.04 **b**	1.25	5.10 **a**	0.58
LSD 0.05		0.54		0.58		0.63	
2005							
CONTROL	-	3.69 **a**	-	3.69 **a**	-	3.50 **a**	-
Calipso 480 SC	0.10	3.79 **ab**	0.10	3.70 **a**	0.01	3.87 **bc**	0.38
Calipso 480 SC + Alert 375 SC	0.10 + 1.20	4.32 **e**	0.63	4.49 **de**	0.80	3.74 **a-c**	0.25
Calipso 480 SC + Horizon 250 EW	0.10 + 1.20	4.05 **b-e**	0.36	4.35 **c-e**	0.66	3.77 **a-c**	0.27
Patriot 100 EC	0.075	3.88 **a-c**	0.19	4.20 **b-d**	0.51	3.57 **ab**	0.07
Patriot 100 EC + Alert 375 SC	0.075 + 1.20	4.04 **b-e**	0.35	4.11 **bc**	0.42	3.98 **c**	0.48
Patriot 100 EC + Horizon 250 EW	0.075 + 1.20	4.25 **e**	0.56	4.56 **e**	0.87	3.94 **c**	0.45
Proteus 110 OD	0.60	3.94 **a-d**	0.24	3.92 **ab**	0.23	3.94 **c**	0.44
Proteus 110 OD + Alert 375 SC	0.60 + 1.20	4.16 **c-e**	0.46	4.34 **c-e**	0.65	3.97 **c**	0.48
Proteus 110 OD + Amistar 250 SC	0.60 + 1.20	4.21 **de**	0.52	4.25 **b-e**	0.56	3.94 **c**	0.44
LSD 0.05		0.30		0.34		0.35	

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 59-71

Differential effect of different nitrogen and sulphur fertiliser regimes on plant health and seed quality of winter oilseed rape grown in Poland

Czesław Sadowski[1], Krzysztof Jankowski[2], Leszek Lenc[1] and Aleksander Łukanowski[1]
[1]Dept. of Phytopathology, University of Technology and Agriculture, Koreckiego 20, 85-225 Bydgoszcz, Poland, e-mail: sadowski@atr.bydgoszcz.pl; [2]University of Warmia and Mazury, Oczapowskiego 2, 10-719 Olsztyn, Poland

Abstract: The effect of a number of doses of nitrogen and sulphur on the healthiness of two winter oilseed rape varieties (open- pollinated 'Gara' and composite hybrid 'Bufallo') was determined. Spring doses of nitrogen were applied at 80, 160 and 240 kg·ha^{-1} and sulphur at 30, 60 and 90 kg·ha^{-1}. Healthiness of plants was evaluated at flowering and during ripening using assessments of infection and then an index was calculated. After harvest the effect of fertilisation on the pathogenic fungal colonisation of seeds was assessed. *Alternaria* spp. were observed at higher levels on leaves and pods and *Phoma ligam* on leaves and stems.

There were observed differences of the healthiness of plants between varieties with the open-pollinated cultivar Gara being more infected. There were complex differential effects of fertilisation. Higher doses of nitrogen resulted in an increase in black spot (*Alternaria* spp.) severity. However, simultaneous fertilisation with nitrogen and sulphur resulted in lower intensity of symptoms of black spot and stem canker. The effect of sulphur fertilisation on plant health was not clear, but the use of this element together with higher doses of nitrogen may show some protective activity against plant infection. The main pathogenic species isolated from seeds was *Alternaria brassicae*. There was no relationship between fertilisation and occurrence of *A. brassicae* on seeds.

Key words: Winter oilseed rape, fertilisation, nitrogen, sulphur, diseases, plant health

Introduction

Similar to most *Brassicaceae,* oilseed rape requires a high level of sulphur. Sulphur requirement significantly affects seed yield and the quality of products obtained from these seeds. Sulphur deficiency results in low number of pods and poor quality seeds. In such situations, the nitrogen use efficiency of the plant and biosynthesis of proteins is also not sufficient. Improper nitrogen assimilation may lead to abnormal development of flowers and siliques (Bilsborrow *et al*., 1995; Mc Grath & Zhao, 1996).

Products of primary and secondary metabolism, e.g. glucosinolates, phytoalexins play an important role in rape pest and disease resistance (Schnug & Haneklaus, 1995). Recently, the use of modern industrial processes has led to a reduction in sulphur emmisions into atmosphere, hence the sulphur content in soil has become relatively low. In many regions of Poland there is a deficit of sulphur and additional sulphur fertilisation programmes are necessary.

Conversly, fertilisation with sulphur may increase the content of glucosinolates in plants. Products of hydrolysis of these compounds show fungistatic activity. This feature can result in lower susceptibility of plants to fungal diseases (Schnug & Ceynowa, 1990; Wallsgrove *et al.,* 1999; Schnug *et al.,* 1995; Drozdowska *et al.,* 2002). Earlier research involving differential

fertilisation of spring oilseed rape with nitrogen and sulphur showed some effect of sulphur on plant health (Sadowski *et al.,* 2000; Drozdowska *et al.,* 2002; Sadowski e*t al.,* 2002; Sadowski *et al.,* 2002a; Sadowski *et al.,* 2003). This paper reports similar experiments with the winter form of rape.

Materials and methods

The experiment was conducted in 2001-2003 under field conditions at the Experimental Station in Balcyny owned by the Department of Crop Production at the University of Warmia and Mazury in Olsztyn.

The experiment was established using a split-plot design with four replications to investigate three factors (1-3).

1. spring dose of nitrogen
2. spring dose of sulphur
3. variety (open-pollinated 'GARA' and composite hybrid 'BUFALLO').

The aim of the experiments was to determine the effect of nitriogen and sulphur on the health of the two varieties of winter oilseed rape. In autumn, 26 kg/ ha of nitrogen was applied to all plots. In spring, nitrogen was also applied at rates of 80 kg/ ha (before the beginning of stem extension), 160 kg/ ha (120 kg before stem extension + 40 kg at the beginning of flower bud development) and 240 kg/·ha (140 kg before stem extension + 100 kg in the beginning of flower bud development). Sulphur was applied once in spring at doses of 30, 60 and 90 kg/·ha + control combination with no sulphur (S_0). Both elements were applied in the form of $(NH_4)_2SO_4$. In combinations with higher doses of nitrogen (160 and 240 kg), nitrogen was applied at the second application timing (beginning of flower bud development) as NH_4NO_3. The soil conditions are presented in Table 1.

Table 1. Decription of soil conditions at the site of an oilseed rape experiment done in 2001–2003 at the Experimental Station in Balcyny of the Department of Crop Production at the University of Warmia and Mazury in Olsztyn.

	Growing season		
	2000/2001	2001/2002	2002/2003
Soil pH (1M KCl)	5.3	5.9	5.9
Nutrient content [mg·kg^{-1} of soil]			
P_2O_5	142	263	206
K_2O	130	125	250
Mg_5	41	83	57
$S\text{-}SO_4$	18.1	15.2	6.7

Observations of plant health were conducted during flowering and at the beginning of ripening. *Alternaria* spp. were noted at high levels on leaves and pods and *Phoma ligam* was observed on leaves and stems. Depending on the pathogen, the proper scales for infection determination were used: 0-4º for *Alternaria* spp. and 0-3º for *Phoma lingam*. Disease Index (DI) was calculated and statistically analysed using analysis of variance and Tuckey's test.

After harvest, the presence of pathogenic fungi on seeds was also assessed. Seeds were washed and put onto blotting paper soaked with 2,4-D (Cappelli *et al.,* 1998). Four hundred seeds were assessed for each treatment combination.

Results and disscusion

Infection of plants was more dependent on year of investigation and cultivated variety than fertilization, however in some years some effects of nitrogen and sulphur treatments were observed (Tab.2).

Table 2. Effect of nitrogen, sulphur and variety on occurrence of *Alternaria* spp. on leaves and pods of winter oilseed rape, Balcyny 2001-2003.

Dose [kg·ha^{-1}], variety	2001	2002	2003	2001-2003	2001	2002	2003	2001-2003
	Leaves				Pods			
Nitrogen								
80	19.1	42.1 **c***	14.4 **b**	25.2	22.4 **ab**	11.4	6.8	13.5
160	21.7	49.4 **b**	18.0 **ab**	29.7	24.1 **a**	11.5	7.2	14.3
240	20.2	52.6 **a**	21.6 **a**	31.5	19.3 **b**	12.6	7.5	13.1
$LSD_{\alpha=0.05}$	n.s.**	2.28	6.73	n.s.	3.33	n.s.	n.s.	n.s.
Sulphur								
0	22.5 **a**	47.3	18.0	29.3	24.3 **a**	14.2 **a**	7.4	15.2
30	20.9 **ab**	48.4	17.3	28.9	21.9 **ab**	10.8 **b**	7.2	13.3
60	17.7 **c**	47.9	18.8	28.1	20.2 **b**	11.8 **b**	6.9	13.0
90	20.0 **bc**	48.5	17.9	28.8	21.4 **b**	10.5 **b**	7.3	13.1
LSD	2.38	n.s.	n.s.	n.s.	2.80	1.87	n.s.	n.s.
Variety								
open-pollinated	22.8 **a**	49.7 **a**	20.6 **a**	31.0 **a**	26.1 **a**	11.8	8.1 **a**	15.4 **a**
hybrid	17.6 **b**	46.4 **b**	15.5 **b**	26.5 **b**	17.7 **b**	11.8	6.3 **b**	11.9 **b**
$LSD_{\alpha=0.05}$	1.54	2.02	1.18	0.91	1.41	n.s.	0.62	2.20

*/ - values in the same column (for every factor separately) followed by different letters are significantly different

**/ - not significant

In two years of investigation, more symptoms of dark leaf spot (*Alternaria* spp.) were observed on plants fertilised with the highest doses of nitrogen. Statistical analysis indicated that over the three years, increased nitrogen rate resulted in higher disease intensity. Infection of pods was statistically significant only in 2001 and in other years as well as in the case of mean for three years there were no significant differences.

The effect of sulphur on occurrence of *Alternaria* spp. was not as clear. In 2001, there were less symptoms on leaves after fertilisation with higher doses of this element, but statistical analysis of mean DI did not show significant differences. Effect of sulphur fertilisation on low levels of infection on pods was observed in two years of investigation. The severity of *Alternaria* spp. and *Phoma lingam* symptoms was higher on the open-pollinated variety in comparison to the hybrid variety.

Table 2a. Interactions between nitrogen and sulphur fertilization and their effect on occurrence of *Alternaria* spp. on leaves and pods of winter oilseed rape, Balcyny 2001-2003

Years	Dose [kg·ha^{-1}]	Leaves			Pods		
		N=80	N=160	N=240	N=80	N=160	N=240
2001	S=0	18.4 a* b	26.6 a a	22.5 a ab	20.0 a b	31.2 a a	21.6a b
	S=30	19.1 a a	23.1 ab a	20.6 ab a	24.1 a a	23.1 b ab	18.4 ab b
	S=60	18.1 a a	17.9 c a	17.2 b a	23.7 a a	21.6 b a	15.3 b b
	S=90	20.6 a a	19.1 bc a	20.3 ab a	21.9 a a	20.3 b a	21.9 a a
	$LSD_{\alpha=0.05}$	A/B**=4.44		B/A=4.13	A/B=5.35		B/A=4.85
2002	S=0	38.3	51.0	52.7	12.1 a a	15.3 a a	15.3 a a
	S=30	40.7	49.0	55.7	10.5 a a	9.7 b a	12.2 ab a
	S=60	44.3	49.3	50.0	11.9 a a	10.3 b a	13.3 a a
	S=90	45.0	48.3	52.3	11.1 a a	10.8 b a	9.4 b a
	$LSD_{\alpha=0.05}$	A/B=n.s.		B/A=n.s.	A/B=n.s.		B/A=3.25
2003	S=0	13.7 a b	18.5 ab ab	21.9 ab a	6.8 ab a	7.5 a a	7.8 ab a
	S=30	15.0 a a	15.0 b a	21.9 ab a	7.8 a a	7.3 a a	6.5 b a
	S=60	13.7 a b	19.1 a ab	23.6 a a	5.5 b b	6.3 a b	8.8 a a
	S=90	15.0 a a	19.5 a a	19.2 b a	7.2 ab a	7.8 a a	7.0 ab a
	$LSD_{\alpha=0.05}$	A/B=7.44		B/A=3.77	A/B=2.09		B/A=1.88
2001 - 2003	S=0	23.5 b b	32.0 a a	32.3 a a	12.8	18.0	14.9
	S=30	24.9 ab a	29.1 ab a	32.7 a a	14.1	13.4	12.4
	S=60	25.4 ab a	28.8 b a	30.3 a a	13.7	12.7	12.5
	S=90	26.9 a a	29.0 ab a	30.6 a a	13.4	13.0	12.8
	$LSD_{\alpha=0.05}$	A/B=7.81		B/A=3.18	A/B=n.s.		B/A=n.s.

*/ - values in the same column and lines (for leaves and pods separately) followed by different letters are significantly different

**/ A – nitrogen, B – sulphur

Taking into consideration simultaneous effect of nitrogen and sulphur on occurrence of dark leaf spot, it was observed that sulphur fertilisation did not have a significant effect on disease severity when nitrogen dose was 80 kg·ha^{-1}. However, analysis conducted for the whole period of research indicated that the highest leaf infection was observed after fertilisation with 90 kg of sulphur (Tab. 2a). Results obtained after fertilisation with sulphur at

various doses of nitrogen (160 and 240 kg·ha^{-1}) were differentiated. The use of 60 kg sulphur resulted in a lower number of disease symptoms.

Investigations over three years indicated that for control treatments (S_0), disease was observed to be more severe at higher doses of nitrogen. Generally, doses of nitrogen and sulphur had no significant effect on disease severity, although some effects were observed in particular years.

Analysis of the effect of nitrogen dose and variety (open-pollinated or hybrid) on occurrence of dark leaf spot, indicated a tendency for leaf infections to decrease with respect to the hybrid variety (Tab. 2b).

Table 2b. Interactions between nitrogen and variety and the effect on *Alternaria* spp. on leaves and pods of winter oilseed rape, Balcyny 2001-2003

Dose [kg·ha^{-1}]	Leaves			Pods		
	N=80	N=160	N=240	N=80	N=160	N=240
2001						
open-pollinated	21.6	24.7	22.2	25.5	28.6	24.4
hybrid	16.6	18.6	18.1	19.4	19.5	14.2
$LSD_{\alpha=0.05}$	A/C**=n.i	C/A=n.s.		A/C=n.s.	C/A=n.s.	
2002						
open-pollinated	43.3	50.5	55.2	10.3	12.1	13.2
hybrid	40.8	48.3	50.1	12.6	11.0	11.9
$LSD_{\alpha=0.05}$	A/C=n.s.	C/A=n.s.		A/C=n.s.	C/A=n.s.	
2003						
open-pollinated	15.6 a* b	21.2 a ab	25.0 a a	7.9	8.0	8.5
hybrid	13.2 b a	14.9 b a	18.3 b a	5.7	6.5	6.6
$LSD_{\alpha=0.05}$	A/C=6.81	C/A=2.05		A/C=n.s.	C/A=n.s.	
2001-2003						
open-pollinated	26.8	32.1	34.1	14.6 a a	16.2 a a	15.4 a a
hybrid	23.5	27.3	28.9	12.5 b a	12.3 b a	10.9 b a
$LSD_{\alpha=0.05}$	A/C=n.s.	C/A=n.s.		A/C=n.s.	C/A=1.25	

*/ - values in the same column and lines (for leaves and pods separately) followed by different letters are significantly different

**/ A – nitrogen, C – variety

In 2003, differences between varieties were significant at all doses of nitrogen applied. Mean DI of pods calculated for the whole period of research was significantly lower at all three doses of nitrogen for the hybrid variety in comparison with the open-pollinated variety, but no differences were noted in particular years of experiments.

Taking into consideration mean DI, there were no significant differences in the levels of infection on leaves of the two varieties with respect to *Alternaria* spp. at various doses of sulphur fertilization, but infection of stems of the open-pollinated variety was statistically higher at each nitrogen dose being applied (Tab. 2c).

Table 2c. Interactions between sulphur and variety and effect on severity of *Alternaria* spp. on leaves and pods of winter oilseed rape, Balcyny 2001-2003

Dose [kg·ha^{-1}]	Leaves				Pods			
	S=0	S=30	S=60	S=90	S=0	S=30	S=60	S=90
2001								
open-pollinated	23.5 a* a	23.7 a a	19.4 a b	24.6 a a	29.0	26.0	24.8	24.8
hybrid	21.5 a a	18.1 b b	16.0 b b	15.4 b b	19.6	17.7	15.6	17.9
$LSD_{\alpha=0.05}$	B/C**=3.20		C/B=3.09		B/C=n.s.		C/B=n.s.	
2002								
open-pollinated	49.8	48.7	49.1	51.1	13.7	12.4	11.1	10.2
hybrid	44.9	48.2	46.7	46.0	14.7	9.3	12.6	10.7
$LSD_{\alpha=0.05}$	B/C=n.s.		C/B=n.s.		B/C=n.s.		C/B=n.s.	
2003								
open-pollinated	20.2 a b	18.9 a b	23.0 a a	20.1 a b	8.9	7.8	8.1	7.8
hybrid	15.7 b a	15.7 b a	14.6 b a	15.7 b a	5.9	6.7	5.7	6.9
$LSD_{\alpha=0.05}$	B/C=2.71		C/B=2.37		B/C=n.s.		C/B=n.s.	
2001-2003								
open-pollinated	31.2	30.4	30.5	31.9	17.2	15.4	14.7	14.3
hybrid	27.4	27.4	25.8	25.7	13.3	11.2	11.3	11.8
$LSD_{\alpha=0.05}$	B/C=n.s.		C/B=n.s.		B/C=n.s.		C/B=n.s.	

*/ - values in the same column and lines (for leaves and pods separately) followed by different letters are significantly different

**/ B – sulphur, C – variety

Stem canker (*Phoma lingam*) was the other pathogen observed at a relatively high severity. For this disease, more disease symptoms were observed on leaves and stems after applications of the higher nitrogen doses. The effect of sulphur on the severity of phoma on leaves and stems as well as mean DI was not clear in study years. Significant differences were observed in infection severity between the two varieties. DI for every year as well as its mean value was statistically higher for leaves of the open-pollinated variety ‘GARA’ in comparison to the hybrid variety. In the case of stems the results were differentiated and depended on year (Tab. 3).

Table 3. Effect of nitrogen, sulphur and variety on occurrence of *Phoma lingam* on leaves and stems of winter oilseed rape, Balcyny 2001-2003

Dose [kg·ha^{-1}], variety	2001	2002	2003	2001-2003	2001	2002	2003	2001-2003
	Leaves				Stems			
Nitrogen								
80	23.8	4.9	4.4	11.0 b*	2.2 b	7.6 b	9.1	6.3
160	26.0	5.4	4.9	12.1 a	3.4 a	8.7 b	9.4	7.2
240	25.0	5.2	4.7	11.7 ab	2.2 b	10.7 a	9.2	7.4
$LSD_{\alpha=0.05}$	n.s.**	n.s.	n.s.	0.82	0.91	1.69	n.s.	n.s.
Sulphur								
0	25.9 ab	5.4 ab	4.9 ab	12.1 ab	2.3 b	9.4	10.2 a	7.3
30	27.4 a	5.6 a	5.1 a	12.7 a	3.5 a	9.3	8.7 b	7.2
60	22.6 c	4.7 c	4.3 c	10.5 b	2.3 b	8.4	9.3 ab	6.6
90	23.9 bc	4.9 bc	4.4 bc	11.1 ab	2.5 b	9.0	8.8 ab	6.8
LSD	2.55	0.59	0.55	2.10	0.66	n.s.	1.41	n.s.
Variety								
open-pollinated	29.1 a	6.0 a	5.5 a	13.5 a	4.0 a	7.8 b	8.4 b	6.7
hybrid	20.8 b	4.3 b	3.8 b	9.7 b	1.2 b	10.2 a	10.1 a	7.2
$LSD_{\alpha=0.05}$	1.49	0.33	0.32	1.59	0.38	0.86	0.77	n.s.

*/ - values in the same column (for every factor separately) followed by different letters are significantly different

**/ - not significant

Taking into consideration simultaneous effect of nitrogen and sulphur on severity of *Phoma lingam* on leaves and stems, no significant differences were observed. However, there was some influence of nitrogen and sulphur in some years of research. For example, in all seasons, at a dose of 240 kg of nitrogen, higher sulphur fertilisation resulted in lower intensity of disease on leaves. It is worth noting that in control combinations of sulphur (S_0) increase of nitrogen doses caused higher incidence of *P. lingam* on leaves and stems (Tab. 3a).

Analysing the effect of nitrogen dose and variety (open-pollinated or hybrid) on occurrence of canker on leaves, there was a tendency for a higher intensity of infection on the open-pollinated variety. This was also observed with respect to mean DI over the three years. This phenomenon was observed for all dose rates of nitrogen. Similar differences were not found for stems (Tab. 3b). With respect to interactions between sulphur fertilisation dose and variety, analysis of results showed no significant effect on leaf infection. In the case of stems it was not clear and highly differentiated (Tab. 3c).

Table 3a. Interactions between nitrogen and sulphur fertilization and their effect on occurrence of *Phoma lingam* on leaves and stems of winter oilseed rape, Balcyny 2001-2003

Years	Dose [kg·ha^{-1}]	Leaves			Stems		
		N=80	N=160	N=240	N=80	N=160	N=240
2001	S=0	21.9 b* b	28.1 a a	27.8 a a	1.9 a b	1.7 c b	3.2 a a
	S=30	24.4 ab b	29.4 a a	28.4 a ab	2.8 a b	6.4 a a	1.2 b c
	S=60	21.3 b a	23.4 b a	23.1 b a	2.1 a b	3.5 b a	7.6 b b
	S=90	27.8 a a	23.1 b b	20.6 b b	2.0 a b	2.1 c ab	3.4 a a
	$LSD_{\alpha=0.05}$	A/B**=4.44		B/A=4.42	A/B=1.33		B/A=1.14
2002	S=0	4.5 b b	5.8 a a	5.8 a a	5.5 b b	10.5 a a	12.2 a a
	S=30	5.1 ab a	5.7 a a	6.1 a a	8.9 a a	8.2 a a	10.7 a a
	S=60	4.5 b a	5.0 a a	4.7 b a	7.1 ab a	7.6 a ab	10.4 a a
	S=90	5.6 a a	4.8 a ab	4.3 b b	8.9 a a	8.4 a a	9.6 a a
	$LSD_{\alpha=0.05}$	A/B=1.07		B/A=1.02	A/B=3.08		B/A=3.00
2003	S=0	4.0 b b	5.3 a a	5.3 a a	8.5 a b	11.8 a a	10.2 a ab
	S=30	4.6 ab a	5.2 ab a	5.5 a a	9.6 a a	8.4 b a	8.2 a a
	S=60	4.0 b a	4.6 ab a	4.2 b a	9.5 a a	8.4 b a	9.8 a a
	S=90	5.0 a a	4.3 b ab	3.9 b b	8.9 a a	8.9 b a	8.6 a a
	$LSD_{\alpha=0.05}$	A/B=0.99		B/A=0.96	A/B=2.27		B/A=2.45
2001 - 2003	S=0	10.1	13.1	13.0	5.3	8.0	8.6
	S=30	11.3	13.4	13.3	7.1	7.7	6.7
	S=60	9.9	11.0	10.7	6.3	6.5	7.1
	S=90	12.8	10.8	9.6	6.6	6.5	7.2
	$LSD_{\alpha=0.05}$	A/B=n.s.		B/A=n.s.	A/B=n.s.		B/A=n.s.

*/ - values in the same column and lines (for leaves and pods separately) followed by different letters are significantly different

**/ A – nitrogen, B – sulphur

Table 3b. Interactions between nitrogen and variety and their effect on occurrence of *Phoma lingam* on leaves and stems of winter oilseed rape, Balcyny 2001-2003

Dose [kg·ha^{-1}]	Leaves			Stems		
	N=80	N=160	N=240	N=80	N=160	N=240
2001						
open-pollinated	28.0	31.2	28.0	3.3 a* b	5.5 a a	3.3 a b
hybrid	19.7	20.8	22.0	1.1 b a	1.4 b a	1.2 b a
$LSD_{\alpha=0.05}$	A/C**=n.s.		C/A=n.s.	A/C=0.99		C/A=0.66
2002						
open-pollinated	5.8	6.4	6.0	6.8	7.1	9.4
hybrid	4.0	4.3	4.5	8.4	10.2	12.0
$LSD_{\alpha=0.05}$	A/C=n.s.		C/A=n.s.	A/C=n.s.		C/A=n.s.
2003						
open-pollinated	5.2	5.8	5.4	8.6	8.3	8.3
hybrid	3.5	3.9	4.1	9.7	10.4	10.1
$LSD_{\alpha=0.05}$	A/C=n.s.		C/A=n.s.	A/C=n.s.		C/A=n.s.
2001-2003						
open-pollinated	13.0 a b	14.5 a a	13.1 a b	6.2	7.0	7.0
hybrid	9.1 b b	9.7 b ab	10.2 b a	6.4	7.4	7.8
$LSD_{\alpha=0.05}$	A/C=1.07		C/A=1.0	A/C=n.s.		C/A=n.s.

*/ - values in the same column and lines (for leaves and pods separately) followed by different letters are significantly different

**/ A – nitrogen, C – variety

The main species occurring on seeds every year was *Alternaria alternata*. Pathogens of rape were represented mainly by *Alternaria brassicae*, which ranged from, 1.2 to 5.5% of seeds depending on year. *Fusarium* spp. were also isolated and rarely *Phoma lingam* (Tab. 4). On the mycelium of *Alternaria* spp., the hyperparasite *Gonatobotrys simplex*, was observd quite often. Apart from species mentioned above, *Botrytis cinerea* and fungi from the genus *Penicillium, Aspergillus, Cladosporium* and *Epicoccum* were also obtained. There was no relationship between fertilisation and fungi occurrence on seeds.

Sulphur fertilisation resulted in better plant health, but there was a differential effect between particular years and combinations. In previous experiments with spring forms of rape, the influence of sulphur was quite distinct (Sadowski *et al.*, 2000; Sadowski *et al.*, 2002). Increased resistance of rape to fungal disease after sulphur fertilisation was reported by Schnug *et al.* (1995) and Booth *et al.* (1995). In experiments conducted by Schnug & Ceynowa (1990), deficiency of sulphur in soil resulted in higher levels of infection by *Cylindrocarpon concentricum*.

Table 3c. Interactions between sulphur fertilization and variety and their effect on occurrence of *Phoma lingam* on leaves and stems of winter oilseed rape, Balcyny 2001-2003

Dose [kg·ha^{-1}]	Leaves				Stems			
	S=0	S=30	S=60	S=90	S=0	S=30	S=60	S=90
2001								
open-pollinated	29.2	31.7	26.9	28.5	2.9 a* c	6.0 a a	3.3 a bc	4.0 a b
hybrid	22.7	23.1	18.5	19.2	1.6 b a	1.0 b a	1.3 b a	1.0 b a
$LSD_{\alpha=0.05}$	B/C=n.s.**		C/B=n.s.		B/C=0.84		C/B=0.77	
2002								
open-pollinated	6.1	6.4	5.8	5.9	7.7 b a	7.0 b a	7.7 a a	8.8 a a
hybrid	4.7	4.8	3.7	3.9	11.1 a ab	11.6 a a	9.1 a b	9.2 a b
$LSD_{\alpha=0.05}$	B/C=n.s.		C/B=n.s.		B/C=2.09		C/B=1.71	
2003								
open-pollinated	5.6	5.8	5.2	5.3	8.9 b a	8.6 a a	8.9 a a	7.3 b a
hybrid	4.2	4.3	3.3	3.5	11.4 a a	8.9 a b	9.6 a b	10.4 a ab
$LSD_{\alpha=0.05}$	B/C=n.s.		C/B=n.s.		B/C=1.76		C/B=1.54	
2001-2003								
open-pollinated	13.6	14.6	12.6	13.3	6.5	7.2	6.6	6.7
hybrid	10.6	10.8	8.5	8.9	8.1	7.1	6.6	6.8
$LSD_{\alpha=0.05}$	B/C=n.s.		C/B=n.s.		B/C=n.s.		C/B=n.s.	

*/ - values in the same column and lines (for leaves and pods separately) followed by different letters are significantly different

**/ B – sulphur, C – variety

Also Walker & Booth (1994) observed positive effect of sulphur on rape health. In the studies presented here, infection of plants was generally higher in combinations with increased doses of nitrogen alone (no sulphur fertilisation). In the case of combinations with fertilisation with both sulphur and nitrogen, better plant health was noted in comparison with combinations fertilised only with nitrogen alone, even at high doses.

A sufficient sulphur content in soil is essential in rape cultivation. This element affects nitrogen uptake, seed and oil yield, composition of fatty acids and glucosionlates. Glucosinolate content may, in some degree, positively influence on plant health, although their role as a part of plant defense system has not been fully described to date (Doughty *et al.,* 1991; Schnug & Ceynowa, 1990; Schnug & Haneklaus, 1995; Giamoustaris & Mithen, 1995; Haneklaus *et al.,* 1999; Wallsgrove *et al.,* 1999; Zhao *et al.,* 1999; Drozdowska *et al.,* 2002). Many authors highlight the need to pay attention to the interaction between nitrogen and sulphur fertilisation in winter rape cultivation and to determine N:S ratio of obtained seed yield (McGrath & Zhao, 1996; Zhao *et al.,* 1995).

The high occurrence of *Alternaria* spp. on seeds every year suggests a need for further investigations on the role of this species in rape pathogenesis, seed health and fodder quality especially in respect to threat by mycotoxins.

Tabele 4. The main fungi occurring on seeds harvested from of winter oilseed rape, Balcyny 2001-2003

Nitrogen [kg·ha^{-1}]	Sulphur [kg·ha^{-1}]	Variety							
		OP*	H**	OP	H	OP	H	OP	H
		Alternaria alternata		*Alternaria brassicae*		*Fusarium* spp.		*Phoma lingam*	
80	0	44,0	43,5	7,5	4,0	7,5	-	-	-
	30	46,0	46,0	1,5	2,5	8,0	5,5	-	-
	60	45,0	47,5	7,0	2,0	1,0	5,0	-	-
	90	47,5	48,0	3,0	5,5	4,5	3,0	-	-
160	0	42,5	44,5	6,0	8,0	-	2,0	0,5	0,5
	30	36,0	45,0	9,5	6,0	5,0	1,0	0,5	1,0
	60	43,5	47,0	3,0	2,0	5,0	-	-	-
	90	42,5	48,0	5,5	4,0	2,5	6,0	0,5	-
240	0	44,0	41,0	6,5	9,5	-	0,5	0,5	-
	30	41,5	43,5	1,0	1,5	-	2,5	-	-
	60	43,5	40,0	2,0	2,0	-	2,0	0,5	0,5
	90	38,0	41,0	5,5	2,0	-	3,0	-	-

*/ OP – open-pollinated
**/ H – hybrid

Conclusions

1. During experiments, symptoms of *Phoma lingam* on leaves and stems and *Alternaria* spp. on leaves and pods were observed at high levels.
2. Infection of plants depended more on variety and year of investigations than on fertilisation. Stems of the open-pollinated variety were significantly more infected in comparison with hybrid one.
3. In two years of investigations, more symptoms of dark leaf (*Alternaria* spp.) were observed on plants fertilised with the highest doses of nitrogen, however fertilisation both with nitrogen and sulphur resulted in lower intensity of disease symptoms.
4. There was no distinct effect of nitrogen fertilisation on symptoms of *Phoma lingam* both on leaves and stems. Some decrease of disease intensity was observed after fertilisation with both sulphur and nitrogen.
5. Influence of sulphur fertilisation on plant health was not clear. However, the use of this element together with higher doses of nitrogen may protect plants against excessive infection.

References

Bilsborrow, P.E., Evans, E.J., & Zhao, F.J. 1995. Changes in the individual glucosinolate profile of double low oilseed rape as influenced by spring nitrogen application. – 9 th Intern. Rapeseed Congress, Cambridge 2: 553-555.

Booth, E.J., Walker, K.C., Taylor, B.R., & Griffiths, D.W. 1995. The influence of foliar glucosinolates in varietal resistance to light leaf spot *(Pyrenopeziza brassicae*). – 9th Intern. Rapeseed Congress, Cambridge 4: 1272-1274.

Cappelli, C., Winter, W., & Paul, V.H. 1998. Detection of seed-transmitted pathogens of rape (*Brassica napus* ssp. *oleifera* D.C.). – IOBC/wprs Bulletin 21 (5): 1-13.

Doughty, K.J., Porter, A.J.R., Morton, A.M., Kiddle, G., Bock, Ch., & Wallsgrove, R. 1991. Variation in the glucosinolate content of oilseed rape (*Brassica napus* L.) leaves. II. Response to infection by *Alternaria brassicae* (Berk) Sacc. – Annals of Applied Biology 118: 469-477.

Drozdowska, L., Szulc, P., Łukanowski, A., & Sadowski, Cz. 2002. Glucosinolate content and pathogenic fungi occurrence in seeds of spring oilseed rape fertilised with sulphur. – Plant Breeding and Seed Science 46 (2): 3-9.

Giamoustaris, A., & Mithen, R. 1995. Modifying leaf glucosinolates in oilseed rape and its effects upon pest and pathogen interactions. – 9th Intern. Rapeseed Congress, Cambridge 4: 1220-1222.

Haneklaus, S., Paulsen, H.M., Gupta, A.K., Bloem, E., & Schnug, E. 1999. Influence of Sulphur Fertilization on Yield and Quality of Oilseed Rape and Mustard. – 10th Intern. Rapeseed Congress, Canberra, CD-ROM, Doc. No. 13: 1-5.

McGrath, S.P., & Zhao, F.J. 1996. Sulphur uptake, yield responses and the interactions between nitrogen and sulphur on winter oilseed rape (*Brassica napus*). – Journal of Agricultural Science. Cambridge 126: 53-62.

Sadowski, Cz., Skinder, Z., & Łukanowski, A. 2000. Effect of sulphur fertilisation on spring rape health status and fungi composition on harvest seeds. – IOBC/wprs Bulletin 23 (6): 71-76.

Sadowski, Cz., Baturo, A., Lenc, L., & Trzciński, J. 2002. Występowanie mączniaka rzekomego (*Peronospora parasitica*/Pers.ex Fr./Fr.) i mączniaka prawdziwego *(Erysiphe cruciferarum* Opitz ex L.Junell) na rzepaku jarym odmiany Star przy zróżnicowanym nawożeniu azotem i siarką. 23 (2): 391-408.

Sadowski, Cz., Jankowski K., Łukanowski A., Lenc., & Trzciński J. 2002a. Health status of spring rape plants as affected by the sowing date and fertilisation with sulphur, boron and magnesium. – IOBC/wprs Bulletin 25 (2): 93-102.

Sadowski, Cz., Łukanowski, A., Lenc, L., & Trzciński, J. 2003. Occurrence of dark leaf and pod spot on spring oilseed rape and fungi composition on harvested seeds depending on differentiated fertilization with sulphur. – 11th Int. Rapeseed Congr. Copenhagen, Denmark 4: 1103-1105.

Schnug, E., & Ceynowa, J. 1990. Phytopathological aspects of glucosinolates in oilseed rape. – J. Agron. & Crop Science, 165: 319-328.

Schnug, E., Haneklaus, S., Booth, E., & Walker, K.C. 1995. Sulphur supply and stress resistance in oilseed rape. – Proc. of the 9th Intern. Rape Congress, Cambridge 1: 229-231.

Schnug, E., & Haneklaus, S. 1995. Sulphur deficiency in oilseed rape flowers - biochemistry and ecological impact. – 9th Intern.Rapeseed Congress, Cambridge 1: 296-298.

Walker, K., & Booth, E.J. 1994. Sulphur deficiency in Scotland and the effects of sulphur supplementation on yield and quality of oilseed rape. – Norwegian J. Agric. Sci., Suppl. 15: 98-104.

Wallsgrove, R., Bennett, R., Kiddle, G., Bartlet, E., & Ludwig-Mueller, J. 1999. Glucosinolate biosynthesis and pest/disease interaction. – 10th Intern. Rapeseed Congress, New Horizons for Old Crop, Canberra, CD-ROM, Doc. No. 393: 1-5.

Zhao, F.J., Evans, E.J., & Bilsborrow, P.E. 1995. Varietal differences in sulphur uptake and utilization in relation to glucosinolate accumulation in oilseed rape. – 9th Intern. Rapeseed Congress, Cambridge 2: 271-273.

Zhao, F.J., Blake-Kalff, M.M.K., Riley, N., Hawkesford, M.J., & McGrath, S.P. 1999. Sulphur Utilisation Efficiency In Oilseed Rape. – 10th Intern. Rapeseed Congress, New Horizons for Old Crop, Canberra, Australia. CD-ROM, Doc. No.301: 1-5.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 73-80

Effects of mineral fertilisation and crop protection schemes on diseases of winter oilseed rape in Poland

Anna Podlesna [1], Malgorzata Jedryczka [2] and Elzbieta Lewartowska [2]
[1] Institute of Soil Science and Plant Cultivation, National Research Institute, Czartoryskich 8, 24-100 Pulawy, Poland; [2] Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland

Abstract: Winter oilseed rape has a long growing season and during this time is attacked by various diseases. At present, the most common way to control diseases is through fungicide application but this is harmful to the environment and decreases crop profitability due to high cost of chemical products. According to the rules of integrated farming, plant cultivation should integrate biological and technological achievements with proper mineral fertilisation. The aim of this work described in this paper was to evaluate oilseed rape healthiness under different chemical protection and fertilisation regimes. Field experiments were done for two years at Baborowko and Grabow Experimental Stations. The first experimental factor was different levels of chemical protection of plants and the second factor was different levels of nitrogen fertilisation at rates that ranged from 0, 40, 80, 120, 160 to 200 kg N/ha. One treatment regime was characterised by applying mineral fertilisation and one fungicide treatment that contained sulphur. The experiments used two oilseed rape varieties: Kana (Grabow) and Lisek (Baborowko). The occurrence of fungal diseases of oilseed rape and their severities were different in both years of experimentation. An increase in chemical protection of oilseed rape caused a decrease in plant infection with most of diseases. However, greater doses of nitrogen fertilisation favoured the development of fungal diseases. Oilseed rape protected by fungicides and fertilized with sulphur showed, in general, lower infection by the main fungal diseases in comparison with treatments with fungicides without sulphur. The larger seed yield of rapeseed was found in the first experiment year, when lower infection of *Phoma lingam* was observed. Oilseed rape variety Kana cultivated at Grabow yielded higher than Lisek cultivated at Baborowko Station.

Key words: Field experiment, weather condition, nitrogen, sulphur, fungal diseases, yield

Introduction

Oilseed rape is attacked by various diseases during the whole growing season and a problem of its healthiness increases as the growing area enlarges and a higher intensity of cultivation results in shorter rotation periods inbetween crops. Diseases, beside other factors, are the cause of yield losses. An economic treatment threshold for pests is about 3-5.5 % of yield loss (Piekarczyk & Wozny 1986) but in some cases losses can reach 10-60 % yield of healthy plants (Korbas *et al*., 2001). The most important factors that can influence the extent of fungal infection without severe effects on the environment are the new, genetically resistant varieties and proper mineral fertilisation of plants. Presently, the most common way to control oilseed rape diseases is the application of an appropriate fungicide. This is a drastic method and there have been recent efforts to decrease the use of chemicals in agriculture. As part of a move towards integrated farming, modern biological and technological methods for plant cultivation should be used. Industrial means of production like mineral fertilisers and pesticides can be used in moderate amounts with the aim to optimise use and maintain the environment in a good condition. However the application of pesticides and fertilisers as well

as crop variety selection is the choice of the farmer, based on farm conditions. The aim of this work was to evaluate different levels of chemical plant protection as well as the effect of sulphur and nitrogen fertilisation on diseases of winter oilseed rape. The role of sulphur in biotic stresses is not fully understood, but there is strong evidence that innate defense mechanisms against plant pathogens are based on sulphur compounds (Schnug & Ceynowa, 1990). The ability of plants to form protective sulphur compounds depends on sulphur nutrition and the form of sulphur in plant tissues. It is known that sulphur fertilisation leads to an increase in the concentration of these compounds in plant organs (Podlesna *et al*., 2003). One of the better understood sulphur-containing defense compounds in plants are the sulphur-rich proteins and of these, the thionins and defensins appear to be the most important (Kruise *et al*., 2005). According to these authors, the effect of these compounds on pathogenic fungi is not clear; it was found however, that these proteins are mostly located in walls of epidermal cells of seeds and leaves. The apoplast is the primary site of contact with a pathogen that would allow for immediate interaction between sulphur-rich compounds produced by plants and compounds produced by pathogens. Another sulphur compound present in plants is glutathione (GSH). The main biochemical function of GSH in infected plants is the production of secondary metabolites such as lignins that strengthen cell walls. Oilseed rape belongs to *Brassicaceae* – a plant family that produces glucosinolates and phytoalexins. It was found that products of glucosinolate breakdown are effective against *L. maculans*, *P. parasitica* and *Alternaria* spp. (Osbourn 1996). However, according to Booth & Walker (1997) glucosinolates do not provide levels of control equivalent to fungicides but their bitter taste serves as a repelent for herbivores and causes retardation in spore germination and mycelium growth in plant tissues. The same opinion concerns phytoalexins, which can limit colonisation by fungal pathogens but do not totally prevent the disease (Bent, 1996). The mineral status of oilseed rape plants that are properly fertilized with sulphur increases the ability for plant defences to prevent fungal attack. According to Hammond & Lewis (1987) and Podlesna (2004), leaves and stems of these plants are richer with calcium and this way more resistant to diseases.

Material and methods

Field experiments were located at two Agricultural Experimental Stations of IUNG: Baborowko (near Poznan, west Poland) and Grabow (near Pulawy, east Poland). The experiments were done over two growing seasons between the years 2000 - 2002. Nitrogen fertilisation was applied at six levels: control without N (0), 40, 80, 120, 160 and 200 kg N/ha. The control included seed dressing and one application of insecticide - regime A (Table 1). Regime B contained full protection against insects whilst regime C had full protection against insects and spring fungicide application against stem canker (*Leptosphaeria maculans*). Regime D had full protection against insects and diseases using sulphur-lacking pesticides and regime E contained the same plant protection treatments as regime D but with additional use of mineral fertilisers and fungicide containing sulphur. Sulphur dose, 80 kg S/ha, was derived from the amounts present in NPK fertilisers applied in the autumn. In the other treatments, NPK fertilisers without sulphur were used. The experiment was done with two replicates in the randomised blocks system. Two winter oilseed rape varieties: Kana (Plant Breeding Strzelce, Poland) and Lisek (Deutsche Saatveredelung, Germany) were used, respectively in Grabow and Baborówko.

Disease assessments were done for the following doses of nitrogen fertilisation: season 2000/2001 0, 80, 120 and 200 kg N/ha and season 2001/2002 – for all six levels of nitrogen. The evaluation was done for 100 randomly chosen plants per each individual plot. The final

disease assessment was performed at crop maturity, just before harvest. Analysis of variance was done with Statgraphics 5.1 software.

Table 1. Regimes of protection treatments used in oilseed rape experiments at Grabow and Baborowko, Poland over two growing seasons between 2000-2002.

Regime	Crop protection	Time of treatment	Commercial name of the product
A – Control	Seed dressing Insecticide	Before sowing spring	Super Homai 70DS Bancol 50 WP
B - Full protection against insects	Seed dressing Insecticide1 Insecticide 2 Insecticide 3	Before sowing Early spring Spring Petal fall	Super Homai 70DS Bancol 50 WP Bancol 50 WP Bulldock 25 EC
C – Full protection against insects and autumn fungicide application	Seed dressing Fungicide 1 Insecticide 1 Insecticide 2 Insecticide 3	Before sowing Early spring Early spring Spring Petal fall	Super Homai 70DS Horizon 250 EW Bancol 50 WP Bancol 50 WP Bulldock 25 EC
D – Full protection against insects and diseases	Seed dressing Fungicide 1 Insecticide 1 Insecticide 2 Fungicide 2 Insecticide 3	Before sowing Early spring Early spring Spring Petal fall Petal fall	Super Homai 70DS Horizon 250 EW Bancol 50 WP Bancol 50 WP Rovral FLO 255 SC Bulldock 25 EC
E – Full protection against insects and diseases with sulphur fertilisation and use fungicide with sulphur	Seed dressing Fungicide with sulphur Insecticide 1 Insecticide 2 Fungicide 2 Insecticide 3	Before sowing Early spring Early spring Spring Petal fall Petal fall	Super Homai 70DS Siarkol K-85 WP Bancol 50 WP Bancol 50 WP Rovral FLO 255 SC Bulldock 25 EC

Results and discussion

The growing seasons differed by temperature and rainfall patterns (Table 2 & 3). The first season had a gradual decrease of temperatures in the autumn and winter, followed by slow increase of temperatures from February or March in Baborówko and Grabow, respectively. The highest rainfall was observed in the autumn and during the spring. The coolest months had the lowest rainfall. In the second season November was cooler and the lowest temperature occurred in December. However, February was unusualy warm, with mean temperature 4.7 ^{o}C and 3.3^{o}C for Baborowko and Grabow, respectively. February had more rainfall and since then, precipitation increased until harvest.

Meteorological conditions had a great influence on healthiness of oilseed rape at both experimental stations (Table 4 & 5). Infections caused by *Phoma lingam* were observed in both seasons but percent of infected plants was lower in the first season as compared to the following, wetter 2001/02 season. During the 2001/2002 season, more fungal diseases were observed. Apart for the infection by *P. lingam*, we have also found *Alternaria* spp., *Botrytis cinerea* and *Sclerotinia sclerotiorum*. Observations confirm earlier studies of Maczynska *et al*. (2001), Kurowski & Budzynski (2003) as well as Korbas & Mrugas (1998) who found that

warm and wet spring conditions are conducive to the development of fungal infections. Wet weather conditions increase fungal infections in the period of flowering of oilseed rape plants.

Table 2. Meteorological data recorded at the Agricultural Experimental Station of IUNG in Baborowko

Month	2000/2001		2001/2002	
	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
IX	12.8	45.6	12.2	123.3
X	12.0	16.0	12.3	26.0
XI	6.3	48.6	3.3	60.2
XII	2.3	46.6	- 1.5	21.8
I	- 0.1	25.4	0.7	51.0
II	0.6	19.2	4.7	44.6
III	2.3	41.4	6.0	47.2
IV	7.9	41.8	8.6	40.6
V	14.2	12.0	16.1	43.8
VI	14.6	75.4	16.5	32.0
VII	19.7	25.6	20.1	28.4

Table 3. Meteorological data recorded at the Agricultural Experimental Station of IUNG in Grabow

Month	2000/2001		2001/2002	
	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
IX	11.6	77.4	12.1	102.2 (47)
X	10.9	10.9	10.7	29.2
XI	5.9	46.2	1.9	25.2
XII	1.3	44.5	- 5.4	12.6
I	- 0.8	36.9	- 1.1	37.7
II	- 1.3	20.8	3.3	52.3
III	0.2	64.4	4.3	30.6
IV	8.1	107.5	8.4	25.5
V	14.0	13.9	17.0	22.1
VI	15.2	67.4	17.4	104.4
VII	20.3	206.4	21.0	105.0

A significant difference in the percentage of infected plants in relation to protection level was found. The greatest infection occurred in the control regime, which contained only a seed dressing and one insecticide treatment in the spring. Application of insecticides and fungicides had a significant effect on lowering the amount of infection. This dependence was evident in the case of all diseases observed in this study. This result is in agreement with the study by Cieslicki & Przybyl (2001) who found that a combined treatment of chemicals that reduce the incidence and severity of insects and fungal diseases gave the best results.

Chemical control of pests which cause feeding injury on leaves and stems of plants and serve as an entry point for subsequent infections had a positive effect on limitation of oilseed rape diseases. Fungicides used in the autumn and in spring limited infection of oilseed rape by

P. lingam, *Alternaria* spp. and *Pyrenopeziza brassicae*. Very high effectiveness of fungicides applied against *S. sclerotiorum* at flowering time was previously found by Weber & Karolewski (2001). Application of fungicides is a very important factor for pod health of rapeseed plants, especially in seasons of high pressure from *B. cinerea* and *Alternaria* spp. According to Maczynska *et al*. (2001), the best control of diseases was obtained when sprays were applied no later then at start of plant flowering.

Table 4. Fungal pathogen infections on oilseed rape plants in an experiment done at Baborowko, Poland.

Experimental factor	2000/2001	2001/2002				
	Phoma lingam	*Phoma lingam*		*Alternaria* spp.	*Botrytis cinerea*	*Sclerotinia sclerotiorum*
	% of infected plants	% of infected plants	Disease severity of infected plants	Disease severity of infected plants		
Protection:						
A	32.4 d*	48.9 b	1.80 c	2.83 b	0.31 b	1.02 bc
B	27.3 c	49.9 b	1.83 c	2.38 b	0.28 ab	0.97 bc
C	23.1 bc	38.6 a	1.29 a	2.35 b	0.24 ab	1.17 c
D	20.9 b	36.6 a	1.25 a	0.95 a	0.19 a	0.87 ab
E	15.8 a	41.8 a	1.54 b	0.60 a	0.21 ab	0.65 a
Fertilisation (kg N/ha):						
0	21.9 a	29.5 a	0.90 a	1.63 ab	0.29 c	0.44 a
40	-	49.6 c	1.93 c	1.71 ab	0.18 a	0.67 ab
80	22.4 a	45.2 bc	1.70 bc	2.18 c	0.28 c	1.00 bc
120	-	49.9 c	1.87 bc	1.58 a	0.22 b	0.80 bc
160	23.4 a	43.3 bc	1.47 bc	1.88 bc	0.27 c	1.10 c
200	27.9 b	41.5 b	1.40 b	1.96 bc	0.23 b	1.61 d

*Numbers in columns marked with the same letters do not differ significantly

Regime E differed from D by the use of sulphur in fungicides and mineral fertilisers. Based on this comparison it was possible to observe a positive effect of this nutrient on reducing disease. For most diseases the lowest infection occurred in the case of additional application of sulphur. The effect was statistically significant for the percent of plants infected by *P. lingam*, both at Baborowko and Grabow in 2000/01 and for lowering the infection by *S. sclerotiorum* in 2001/02 at Grabow.

Oilseed rape plants fertilised with high doses of nitrogen were more severely infected by *P. lingam*, *S. sclerotiorum* and *Alternaria* spp. at both locations, which was consistent with previous studies (Jedryczka *et al*., 2002). According to Lipa (1992) greater tillering and foliage of plants which are well supplied with nitrogen results in a greater plant density, what creates better conditions for the development of diseases. In contrast, Jankowski & Budzynski (1997) found that a good supply of nitrogen to oilseed rape resulted in lower infection by stem rot caused by *S. sclerotiorum*. In our experiment the effect of nitrogen on severity of *B. cinerea* was not clear, most probably as a result of low disease incidence and severity of plant infection by grey mould during the experiment. The experiments by Jankowski & Budzynski (1997) and Kurowski & Budzynski (2003) showed greater infections in treatments fertilised

with higher doses of nitrogen. Seed yields of oilseed rape were significantly different depending on protection level, nitrogen dose and site (Figures 1 & 2).

Table 5. Fungal pathogen infections on oilseed rape plants in Grabow

Experimental factor	2000/2001	2001/2002				
	Phoma lingam	*Phoma lingam*		*Alternaria* spp.	*Botrytis cinerea*	*Sclerotinia sclerotiorum*
	% of infected plants	% of infected plants	Disease severity of infected plants	Disease severity of infected plants		
Protection:						
A	20.3 c	53.4 c	2.14 c	0.59 c	0.03 a	0.00 a
B	11.3 a	46.9 b	1.83 bc	0.67 c	0.09 c	0.03 b
C	12.9 b	45.4 b	1.73 ab	0.46 b	0.09 c	0.04 bc
D	12.0 b	38.8 a	1.53 ab	0.38 ab	0.05 b	0.08 d
E	9.3 a	37.4 a	1.42 a	0.30 a	0.04 b	0.05 c
Fertilisation (kg N/ha):						
0	8.3 a	29.3 a	1.08 a	0.41 a	0.03 a	0.00 a
40	-	46.0 bc	1.61 b	0.39 a	0.08 b	0.04 cd
80	14.0 b	47.8 c	1.88 bc	0.48 ab	0.09 b	0.03 bc
120	-	43.5 b	1.70 b	0.54 bc	0.02 a	0.09 e
160	14.4 b	51.9 d	2.05 c	0.43 ab	0.04 a	0.05 d
200	14.2 b	48.1 c	2.04 c	0.63 c	0.10 b	0.02 b

*Numbers in columns marked with the same letters do not differ significantly

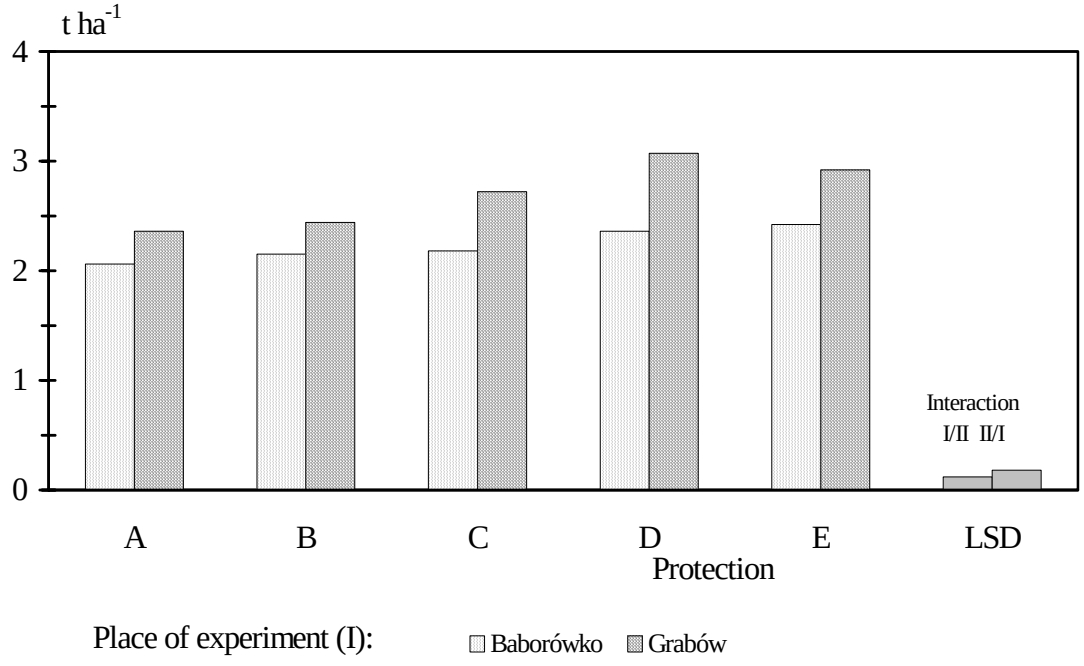


Figure 1. Yield of oilseed rape in oilseed rape experiments with five crop protection regimes at Grabow and Baborowko, Poland during two growing seasons between 2000-2002.

Yields obtained in Baborowko Station were lower than those at Grabow regardless of protection and nitrogen level. The disease incidence and severity was greater in Baborowko than in Grabow. The disease severity caused by *Alternaria* spp., *B. cinerea* and *S. sclerotiorum* was greater in the 2001/2002 season. The use of chemicals and mineral sulphur reduced the epidemics of all diseases and created better conditions for plant growth. Decrease of disease epidemics had a strong effect on yield because leaves and stems of plants infected by S. sclerotiorum (April and May) and then by Alternaria spp. were less able to photosynthesise. At this time oilseed rape plants form buds and enter a flowering period. The colonisation of plants by pathogenic fungi leads to disturbances in their water management, respiration and photosynthesis (Grzesiuk & Koczowska, 1999). Doughty *et al*. (1996) found that fungicide treated plots were characterised by less severe epidemic of diseases and in a response produced dry matter yields up to 1 t ha^{-1} (25%) higher than those from infected plots. A positive effect of nitrogen on oilseed rape yield can be observed in Figure 2. Oilseed rape requires high doses of nitrogen and plants in a good condition can better compensate for damage caused by pests.

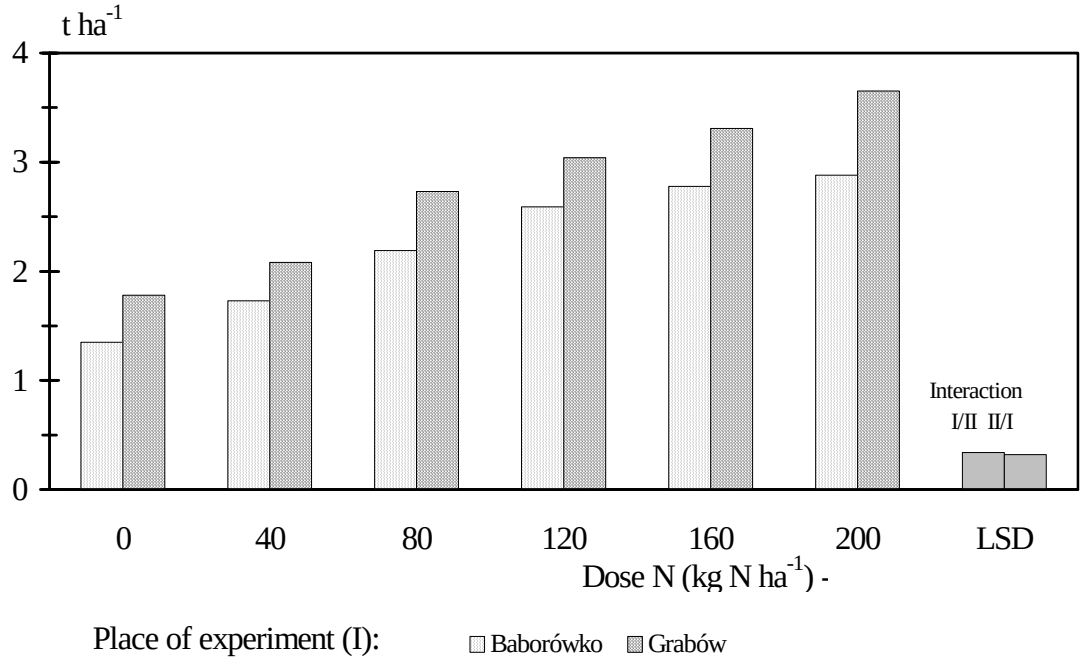


Figure 2. Yielding of oilseed rape with respect to nitrogen dose in oilseed rape experiments at Grabow and Baborowko, Poland during two growing seasons between 2000-2002

References

Bent, A.F. 1996. Plant disease resistance genes: function and structure. – Plant Cell 8: 1757-1771.

Booth, E. & Walker, K. 1997. The effectiveness of foliar glucosinolate content raised by sulphur application on disease control in oilseed rape. – In: W.J. Cram et al. (ed.) Sulphur metabolism in higher plants. Buckhuys Publ., Leiden, The Netherlands: 327-329.

Cieslicki, W. & Przybyl, H. 2001. Effect of the pest control on the phytosanitary state of winter oilseed rape. – Progress in Plant Protection. Vol. 41 (2): 428-431(in Polish).

Doughty, K.J., Lewis, C.J., McCartney, H.A., Norton, G., Booth, E.J. & Walker, K.C. 1996. Oilseed rape yield and quality in relation to fungal disease. – Book of Abstracts 4th ESA- Congress. Veldhoven-Wageningen, The Netherlands 2: 540-541.

Hammond, K. & Lewis, B.G.1987. Variation in stem infestations caused by aggressive and non-aggressive isolates of *Leptosphaeria maculans* in *Brassica napus* var. *oleifera*. – Plant Pathol. 36: 53-65.

Jankowski, K. & Budzynski, W. 1997. Nitrogen fertilization and degree of winter oilseed rape damage by pests. – Zeszyty Problemowe Postepow Nauk Rolniczych 439: 273-280 (in Polish).

Jedryczka, M., Podlesna, A. & Lewartowska, E. 2002: Effect of fertilization with nitrogen and sulphur on healthiness of oilseed rape. – Pamietnik Pulawski 130(I): 329-338 (in Polish).

Lipa, J.J. 1992. The effect of mineral fertilization on occurring of diseases and insects of plants. – Postepy Nauk Rolniczych 2: 29-38 (in Polish).

Korbas, M. & Mrugas, D. 1998. The oilseed rape is frequently attack by diseases. – Top Agrar Polska 4: 54-59 (in Polish).

Korbas, M., Walkowski, T. & Gwiazdowski, R. 2001. Thresholds of harmfullnes of winer oilseed rape diseases. – Progress in Plant Protection 41 (1): 286-290 (in Polish).

Kruse, C., Jost, R., Hillebrand, H. & Hell, R. 2005. Sulfur-rich proteins and their agrotechnological potential for resistance to plant pathogens. – Landbauforschung Völkenrode, Special Issue 283: 73-79.

Kurowski, T.P. & Budzynski, W. 2003. The effect of differentiated fertilization with nitrogen and protection against pests on healthiness of spring oilseed rape. – Oilseed Crops 24 (2): 455-463 (in Polish).

Maczynska, A., Glazek, M., Krzyzinska, K. & Balachowska, J. 2001. Infection of winter oilseed rape by disease fungi in the years 1999 and 2000. – Oilseed Crops, 22 (1): 127-138 (in Polish).

Maczynska, A., Krzyzinska, B. & Drzewiecki, S. 2001. Influence of different terms of application of fungicides on heath of winter rape pods. – Progress in Plant Protection. 41 (2): 638-642 (in Polish).

Osbourn, A.E. 1996. Performer antimicrobial compounds and plant defense against fungal attack. – Plant Cell 8: 1821-1831.

Piekarczyk, K. & Wozny, J. 1986. Thresholds of economical harmfulness of diseases and pests of crops. – Institute of Plant Protection, Poznan: 3-9 (in Polish).

Podlesna, A. 2004. The effect of sulfur fertilization on concentration and uptake of nutrients by winter oilseed rape. – Oilseed Crops 25 (2): 627-636 (in Polish).

Podlesna, A., Jedryczka, M. & Lewartowska, E. 2003. Healthiness of winter oilseed rape fertilized with nitrogen and sulfur. – In: Sulfur transport and assimilation in plants. Ed. J.-C. Davidian et al., Buckhuys Publishers, Leiden, The Netherlands: 307-308.

Weber, Z., & Karolewski, Z. 2001. Influence of some selected triazole fungicides on winter oilseed rape growth and their protection against pathogenic fungi. – Progress in Plant Protection 41 (2): 773-775 (in Polish).

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 81-88

Influence of fertilisation with sulphur, magnesium and boron on the content of glucosinolates and occurence of *Alternaria* spp. on seeds of the spring oilseed rape 'Margo'

Anna Figas[1], Aleksander Łukanowski[2], Lucyna Drozdowska[1] and Czesław Sadowski[2]
[1] *University of Technology and Agriculture, Department of Plant Physiology, ul. Bernardyńska 6, 85-029 Bydgoszcz, Poland*
[2] *University of Technology and Agriculture, Department of Phytopathology, ul. Kordeckiego 20, 85-225 Bydgoszcz, Poland*

Abstract
Effects of soil or foliar fertilisation with sulphur as well as in combination of both together with foliar application with magnesium or boron on the content of glucosinolates and infection of seeds of the spring oilseed rape 'Margo' with *Alternaria* spp. were studied. Laboratory investigations were performed on seeds of oilseed rape of a three-year-long field experiment. The experiment was carried out in 2001-2003 using a design of randomised sub-blocks in 4 replications. It was shown that fertiliser treatments used did not significantly affect the total content of glucosinolates in the seeds. A high negative correlation between concentration of alkenyl glucosinolates, such as progroitrin and glucobrassicanapin, in the seeds and resistance toward the fungal pathogen *Alternaria brassicae* was shown. However, no significant correlation was observed between glucosinolates level and the infestation with the non-pathogenic *A. alternata* species.

Key words: Rape, diseases, fungi, seeds, glucosinolates, fertilisation, boron, magnesium, nitrogen, sulphur

Introduction

Similar to most *Brassicaceae,* oilseed rape requires a high level of sulphur (Schnug & Haneklaus, 1995). Sulphur is important in protecting oilseed rape from numerous diseases (Schnug & Ceynowa, 1990; Pałosz, 1995; Sadowski *et al.,* 2000; Jędryczka *et al.,* 2002). Sulphur is a component of glucosinolates, which may act against numerous insects and pathogens, as well as other antagonistic and symbiotic organisms (Schnug & Ceynowa, 1990; Waligórska & Krzymańska, 1993; Giamoustaris & Mithen, 1995; Wallsgrove *et al.* 1999). There is substantial evidence that isothiocyanates are the most active among the products of glucosinolate hydrolysis (Greenhalgh & Mitchell, 1976; Mithen *et al.,* 1986; Mari *et al.,* 1993; Angus, 1994; Mayton *et al.*, 1986). It has also been reported that some products of glucosinolate hydrolysis inhibited the development of *Peronospora parasitica* (Greenhalgh & Mitchell, 1976), *Leptosphaeria maculans* (Mithen *et al.,* 1986), and *Alternaria* spp. (Milford *at al.,* 1989), however, results are still not fully conclusive. Therefore, the major objectives of this research were: 1) to determine the effects of sulphur, magnesium, and boron on the content of glucosinolates in oilseed rape seeds and 2) to determine a correlation between the level of glucosinolates in oilseed rape seeds and the rate of their infection by *Alternaria* spp.

Materials and methods

The experiment was done at the Chrząstowo Experimental Station of Cultivar Assessment in north-central Poland in 2001, 2002 and 2003 on brown soil from silty light loam and qualified as good and very good wheat complex. The previous crops were small grain cereals. Before treatments were applied, soil was relatively low in sulphur (6.03 mg $SO_4 \cdot kg^{-1}$) and boron (0.71 mg·kg $^{-1}$) and moderate in magnesium (40.7 mg·kg $^{-1}$). The experiment received standard fertilisation with 130 kg N, 50-80 kg P_2O_5, and 70-92 kg K_2O per hectare. Spring oilseed rape *Brassica napus* 'Margo' was fertilised for each year with sulphur, boron and magnesium. The experimental design used was a randomized complete block with 4 replications. The treatments included: sodium sulphate (Na_2SO_4) applied to either the soil or foliage, sodium sulphate supplemented with boron (borax), sodium sulphate supplemented with magnesium sulphate ($MgSO_4 \cdot 7H_2O$), and a control (no fertilisation with sulphur, boron and magnesium). Application method, timing, and rates are presented in Table 1.

Table 1. Application method, timing, and rates used during a series of experiment done at the Chrząstowo Experimental Station of Cultivar Assessment in north-central Poland in 2001, 2002 and 2003

Objects	Element	Fertilizer	Application method	Rate [kg· ha^{-1}]	Time of fertilisation
I	Sulphur	Na_2SO_4	Soil application	40.0	Before sowing
II	Sulphur	Na_2SO_4	Foliar application	20.0	Rosette phase
				20.0	and budding
III	Sulphur	Na_2SO_4	Soil application	40.0	Before sowing
	Boron	borax	Foliar application	0.5	Budding
IV	Sulphur	Na_2SO_4	Soil application	40.0	Before sowing
	Magnesium	$MgSO_4$	Foliar application	1.0	Budding

Content of glucosinolates in seeds was determined using gas chromatography (Michalski *et al.,* 1995) and reported in μmol·g^{-1} of dry matter as disulphoglucosinolates. A filter paper test was used to determine the level of seed infection. Four replicates of 100 seeds from each fertiliser combination were placed into Petri dishes with filter paper moistened using sterile water. Petri dishes were kept for 24 hrs at 20^o C under UV light, followed by 24 hrs of dark conditions at -24°C. Fungi were incubated for 11 days in 20°C at 12h alternating light regime of UV light and dark conditions. Mycological keys were used to assess species of isolated fungi. Results of glucosinolate content in seeds were statistically analyzed with Tukey's test at P= 0.05. The Statistica software was used to conduct regression analysis in the case of the investigation of a relationship between glucosinolate content in seeds and occurrence of *Alternaria* spp.

Results and discussion

Total glucosinolates, alkenyl and indole glucosinolates were not affected by fertilisation with sulphur, sulphur supplemented with boron, and sulphur supplemented with magnesium (Table 2). However, all fertilisation treatments showed a consistent trend toward higher values of more harmful alkenyl glucosinolates. The ratio between alkenyl glucosinolates and

indole glucosinolates has been shifted toward alkenyl glucosinolates and increased by 1 to 4% (Figure 1). The ratio between alkenyl glucosinolates and indole glucosinolates and their share in total content of glucosinolates was similar to results obtained for 'Bolko' by Wielebski *et al.* (1999). In our experiments the highest content of alkenyl glucosinolates was noted after soil application of sulphur and it was mainly caused by an increase in progoitrin level. Similarly, Zhao *et al.* (1995) and Wielebski & Wójtowicz (2003) reported elevated levels of alkenyl glucosinolates as a result of sulphur fertilisation.

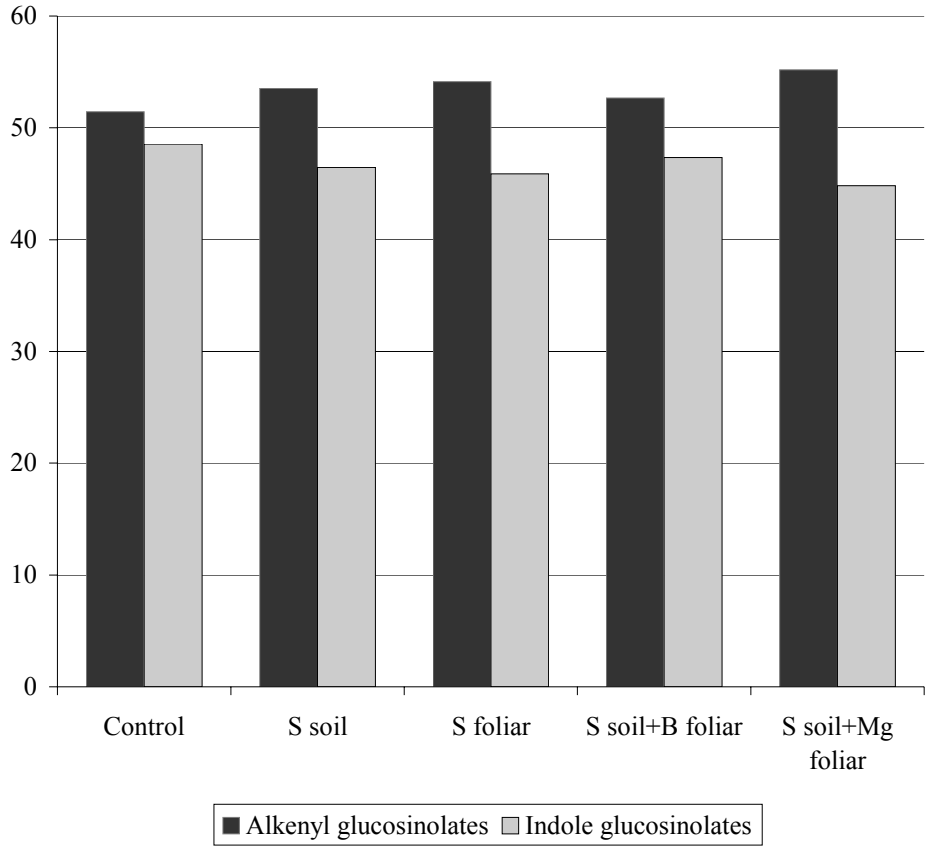


Figure 1. Influence of sulphur, magnesium and boron fertilisation on the content of alkenyl and indole glucosinolates [%] in 'Margo' spring oilseed rape seeds (average over 2001-2003) for an experiment done at the Chrząstowo Experimental Station of Cultivar Assessment in north-central Poland.

Table 2. Influence of sulphur, magnesium and boron fertilisation on the glucosinolate contents in 'Margo' spring oilseed rape seeds (average over 2001-2003). PRO - progoitrin, GNP - gluconapin, GBN – glucobrassicanapin, NPL -napoleiferyne

Object		Content of the glucosinolates in seeds [µmol·g^{-1} d.m.]					
		Total glucosinolates	*PRO*	*GNP*	*GBN*	*NPL*	Total alkenyl glucosinolates
Control		9.82	3.08 b	1.6	0.2	0.17	5.05
Fertilized	*S-soil application*	11.05	3.75 a	1.78	0.28	0.1	5.92
	S-foliar application	10.1	3.35 b	1.77	0.23	0.12	5.47
	S-soil application +B-foliar application	10.32	3.37 b	1.72	0.27	0.08	5.43
	S-soil application +Mg-foliar application	9.97	3.35 b	1.83	0.23	0.08	5.5
Mean		10.25	3.38	1.74	0.24	0.11	5.47
Mean for fertilisation		10.36	3.45	1.78	0.25	0.1	5.58
LSD $_{(\alpha=0,05)}$		n.s.	0.37	n.s.	n.s.	n.s.	n.s.

n.s. – difference not significant

Occurrence of *Alternaria* spp. on seeds showed strong negative correlation between total amount of glucosinolates and seed resistance to *Alternaria brassicae* (Figure 2).

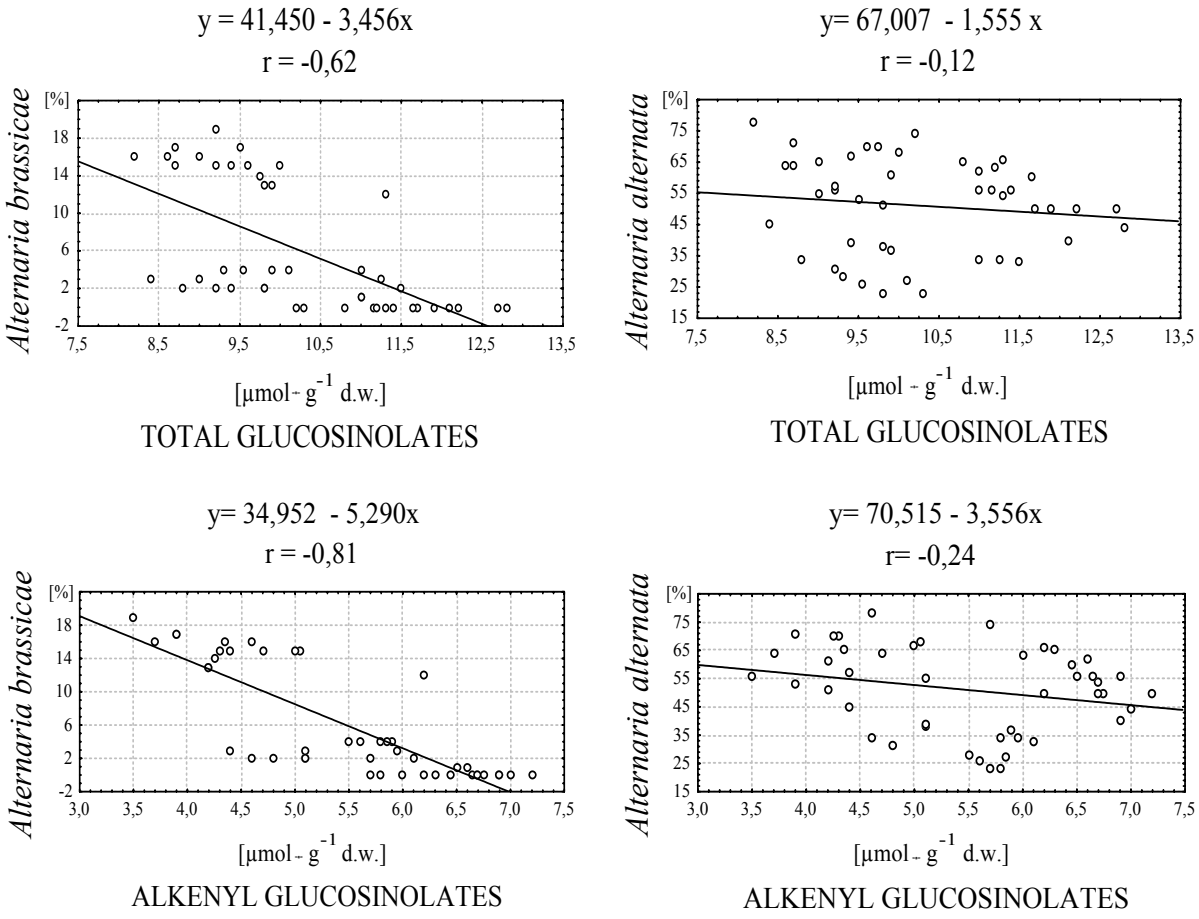


Figure 2. Relationship between the total and alkenyl glucosinolates content in seeds and infection level of *Alternaria* spp. (average over 2001-2003) for an experiment done at the Chrząstowo Experimental Station of Cultivar Assessment in north-central Poland.

Furthermore, it was determined that alkenyl glucosinolates such as progoitryn and glucobrassicanapine were responsible for the increased resistance of seeds while indole glucosinolates were of minor importance (Figure 3). These findings were not consistent with a report on spring oil seed rape 'Star' fertilized with sulphur applied through the foliage and soil (Drozdowska *et al.*, 2002). In this case, it was found that increased resistance of seeds to *A. brassicae* could be attributed to an elevated content of indole glucosinolates, specifically 4-hydroxyglucobrassicine and glucobrassicine.

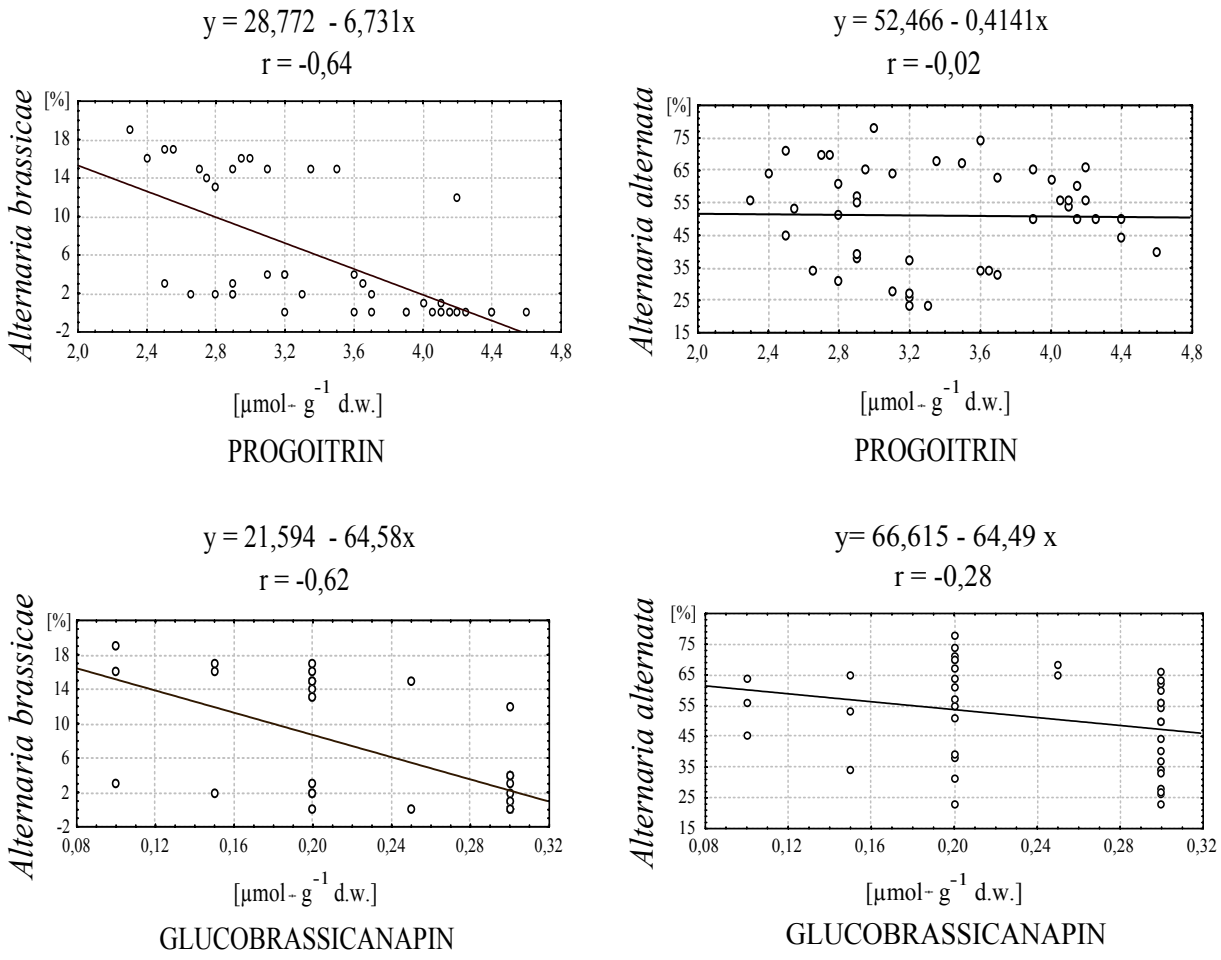


Figure 3. Relationship between alkenyl glucosinolate content in seeds and infection level of *Alternaria* spp. (average over 2001-2003) for an experiment done at the Chrząstowo Experimental Station of Cultivar Assessment in north-central Poland.

These differences could arise from different application rates of sulphur and/or from the natural content of glucosinolates that are specific for a particular variety. Application rates used by Drozdowska *et al.* (2002) were 20 and 60 kg·ha $^{-1}$ while the rates used during the current study were 40 kg·ha $^{-1}$ for both foliar and soil applications. The average content of glucosinolates for 'Star' used by Drozdowska *et al.* (2002) was 30% higher compared with 'Margo' used in our experiment (COBORU, 2004). The role of glucosinolates in governing plant disease resistance is still unclear. Some reports suggest that plants with a low level of glucosinolates are more susceptible to fungal diseases (Mithen *et al.,* 1987; Giamoustaris & Mithen, 1995). Some reports suggest that oilseed rape varieties influence plant disease resistance. Wallsgrove *et.al* (1999) found elevated contents of glucosinolates in leaves after infection of resistant varieties by *Sclerotina sclerotiorum* and no reaction when varieties were susceptible. Giamoustaris & Mitchen (1995) showed that higher contents of glucosinolates in pods reduced their infection by *A. brassicae*. Others reported that a high level of glucibrassicapine did not decrease plant infection with *L. maculans* (Mithen & Magrath, 1992) and elevated level of progoitrine did not inhibit growth of *L. maculans in vitro* (Mithen *et al*., 1986).

Alternaria alternata, is admittedly not pathogenic on oilseed rape, but may also reduce quality of feed by inducing levels of certain mycotoxins. Our results showed no correlation between glucosinolate levels and seed infection by *A. alternata* (Fig. 2 and Fig 3).

Conclusions

1. It was observed that glucosinolate content increased after fertiliser treatments were applied.
2. A significantly higher progoitrin content was found in seeds from plants fertilised with sulphur applied into soil.
3. There was a significant negative correlation between alkenyl glucosinolate content in seeds and their resistance against *A. brassicae*.
4. There was no significant correlation between glucosinolate content and seed infection by *A. alternata*.

References

Angus, J.F. 1994: Biofumigation: isothiocyanates released from *Brassica* roots inhibit of the take-all fungus. – Plant Soil. 162: 107-112.

COBORU 2004: Lista opisowa odmian. – Rośliny rolnicze cz. I.

Drozdowska, L. Szulc, P. Łukanowski, & A. Sadowski, C. 2002: Glucosinolate content and pathogenic fungi occurrence in seeds of spring oilseed rape fertilized with sulphur. – Plant Breeding Seed Sci. 46 (2): 3-9.

Giamoustaris, A. & Mithen, R. 1995: The effect of modifying the glucosinolate content of leaves on oilseed rape (*Brassica napus* spp. *oleifera*) and its interaction with specialist and generalist pest. – Ann. Appl. Biol. 126: 347-363.

Greenhalgh J.R. & Mitchell N.D. 1976: The involvement of flavour volatiles in the resistance to downey mildew of wild and cultivated forms of *Brassica oleracea*. – New Phytologist 77: 391-398.

Jędryczka, M. Podleśna, A. & Lewartowska, E. 2002: Wpływ nawożenia azotem i siarką na zdrowotność roślin rzepaku ozimego. – Pamiętnik Puławski 130: 329-337.

Mari, M., Iori, R., Leoni, O. & March, A. 1993: In vitro activity of glucosinolate-derived isothiocyanates against postharvest fruit pathogens. – Ann. Appl. Biol. 123: 155-164.

Mayton, H.S., Olivier, C., Vaughn, S.V. & Loria, R. 1996: Correlation of fungicidal activity of Brassica species with allyl isothiocyanate production in macerated leaf tissue. – Phytopathology 86: 267-271.

Michalski, K., Kołodziej, K. & Krzymański, J. 1995: Quantitative analysis of glukosinolates in seeds of oilseed rape – effect of sample preparation on analytical results. – 9th International Rapeseed Congress, Cambridge 3: 911-913.

Milford, G.F.J., Fieldsend, J.K., Porter, A.J.R., Rawlinson, C.J., Evans, E.J. & Bilsborrow, P. 1989: Changes in glucosinolate concentrations during the vegetative growth of single and double low cultivars of winter oilseed rape. – Asp. Appl. Biol. 23: 83-90.

Mithen, R.F., Levis, B.G., & Fenwick, G.R. 1986: In vitro activity of glucosinolates and their products against *Leptosphaeria maculans*. – Trans. Brit. Mycol. Soc. 87: 433-440.

Mithen, R.F., Levis, B.G., Heaney, R.K. & Fenwick, G.R. 1987: Resistance of *Brassica* species to *Leptosphaeria maculans* (stem canker) – the role of glucosinolates. – 7th International Rapeseed Congress, Poznań 1: 125-130.

Mithen, R.F., & Magrath, R. 1992: Glucosinolates and resistance to *Leptosphaeria maculans* in wind and cultivated *Brassica* species. – Plant Breed. 108: 60- 68.

Pałosz, T. 1995: Siarka a problemy fitosanitarne w uprawie rzepaku ozimego. – Ochr. Rośl. 12: 5-6.

Sadowski, Cz. Skinder, Z. & Łukanowski, A. 2000: Effect of sulphur fertilization on spring rape health status and fungi composition on harvested seeds. – IOBS/WPRS Bulletin 23 (6): 71-76.

Schnug, E. & Ceynowa, J. 1990: Phytopathological aspects of glucosinolates in oilseed rape. – J. Agron. & Crop Science 165: 319-328.

Schnug, E. & Haneklaus, S. 1995: Sulphur deficiency in oilseed rape flowers-sympto-matology, biochemistry and ekological impact. – 9th International Rapeseed Congress, Cambridge: 296-298.

Waligórska, D. & Krzymańska, J. 1993: Aktywność biologiczna glukozynolanów wyizolowanych z liści rzepaku. – Post. Nauk Rol. 5: 151-156.

Wallsgrove, R., Bennet, R., Kiddle, G., Bartlet, E. & Ludwig-Muller, J. 1999: Glucosinolate biosynthesis and pest/disease interactions. – 10th International Rapeseed Congress, Canberra, Proc. CD Rom. Doc. No. 393: 1-5.

Wielebski, F., Wójtowicz, M. & Krzymański, J. 1999: Influence of sulphur fertilization on glucosinolate quality and quantity in seed of two double low oilseed rape varieties (*Brassica napus* L.). – 10th International Rapeseed Congress, Canberra, Australia CD ROM, Doc. No. 301: 1-5.

Wielebski, W. & Wójtowicz, M. 2003: Wpływ wiosennego nawożenia siarką na plon i zawartość glukozynolanów w nasionach odmian mieszańcowych złożonych rzepaku ozimego. – Rośliny Oleiste 24: 107-119.

Zhao, F., Evans, E.J. & Bilsborrow, P.E. 1995: Varietal differences in sulphur uptake and utylization in relation to glucosinolate accumulation in oilseed rape. – 9th International Rapeseed Congress, Cambridge: 271-273.

ENTOMOLOGY PAPERS

Pest damage to oilseeds – Problems

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 91-94

Insect problems in European oilseed rape cultivation, and how to deal with them: The OSR farmers' perspective

Ingeborg Menzler-Hokkanen[1], Heikki M.T. Hokkanen[2], Wolfgang Büchs[3], Zdzislaw Klukowski[4], Anne Luik[5], Christer Nilsson[6], Bernd Ulber[7] and Ingrid H. Williams[8]

[1] *University of Helsinki, Ruralia Institute, Lönnrotinkatu 3-5, FIN-50100 Mikkeli, Finland*
[2] *University of Helsinki, Department of Applied Biology, Box 27, FIN-00014, Finland*
[3] *BBA, Institute for Protection in Field Crops & Grassland, Messeweg 11/12, D-38108, Braunschweig, Germany*
[4] *Agric.Univ. Wroclaw, Department of Crop Protection, 50-205 Wroclaw, Cybulskiego str. 32, Poland*
[5] *Estonian Agricultural University, Kreutzwaldi 64, Tartu EE 51017, Estonia*
[6] *Swedish University of Agricultural Sciences, P O Box 44, S-230 53 Alnarp, Sweden*
[7] *Georg-August University, Institute for Plant Pathology & Crop Protection, Grisebachstr. 6, D-37077 Göttingen, Germany*
[8] *Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK*

Abstract: A survey was carried out among oilseed rape growers in six countries (Estonia, Finland, Germany, Poland, Sweden and the UK) concerning the growing season of 2002-2003, as part of the EU-project MASTER. In total, 1005 replies were obtained with 115-216 responses from each of the countries surveyed. Here the core results concerning the occurrence and importance of insect pests, the decision making criteria concerning the need for active control measures, and the impact of farming methods (conventional-integrated-organic) on these perspectives are presented. Europe-wide, only the pollen beetle was a problem requiring control in each country. The seed weevil, pod midge, and the cabbage stem flea beetle were the second most commonly mentioned as problems (important in 4 out of 6 countries), followed by the stem weevils (2-3 out of 6 countries). Six-seven different insect species were all very important in DE and PL, while in the UK 2-3 species and in SE, FI and EE only 1-2 species were problematic. This was reflected in the number of insecticide spray treatments: typically 2-4 per season in Poland, 1-4 in Germany, 0-3 in the UK, SE, FI, and EE. Paradoxically, the mean number of insecticide sprays was the same for conventional and IPM farmers (1.65 sprays/season), and in FI and PL farmers using IPM sprayed more often than conventional farmers. Also, the proportion of IPM farmers was highest in countries where the spraying rate also was highest (DE, PL, UK). Furthermore, overall, a higher proportion of IPM farmers used insecticide seed dressing than conventional farmers (77.3% vs. 69.7%). In all countries, the majority of farmers always sprayed all their OSR fields (81.0%), while overall, 14.7% sprayed some but not all fields, and a mere 4.3% reported having sprayed field edges only. Out of nine different decision-making criteria for determining whether active pest control was necessary, farmers were using most often simply seeing the pest on the crop (70.0%), followed by using economic thresholds (47.3%), recommendation by crop protection consultant (24.0%), plant growth stage (16.3%), advisory service message (16.3%), advisory service general recommendation (8.7%), advice from neighbours (8.2%), commercial decision support system (2.7%), or spraying by date (1.8%). Growers in PL used on the average 4.6 different criteria simultaneously to arrive at pest control decision, while in other countries clearly fewer criteria were sufficient: 2.7 in DE, 1.9 in SE and UK, and 1.6 in EE and FI.

Key words: Pest spectrum, pesticide use, decision making, economic threshold, integrated pest management, IPM, survey, EU, conventional farming, organic farming, cross-country comparisons

Introduction

The management of pests on the European oilseed rape (OSR) crop still relies heavily on chemical pesticides, often applied routinely and prophylactically without regard to pest incidence, and at best according to threshold values of the pest population. This has led to over-use of chemical pesticides, reducing the economic competitiveness of the crop and threatening biological diversity.

Detailed information, however, on farmers' current pest management practices in various European countries is lacking. To our knowledge, after Bromand (1990) no cross-European survey on the topic has been carried out; therefore, we conducted a baseline survey in the countries participating in the EU-project MASTER (see Williams *et al*., this issue).

Materials and methods

A questionnaire for OSR growers was drafted jointly by MASTER partners. It had 24 questions concerning growers' current cropping practices, their knowledge of and attitudes towards IPM, and their willingness to change husbandry practices. Background questions, as possible explanatory variables, provided information about the respondent (e.g. owner, employee/operator, tenant, age category), the farm (e.g. size, surrounding habitats, cropping), size of OSR fields and crop rotation practices. Each question had several sub-questions, so that in total 80 separate replies were obtained from each farmer. The practical questions related to the 2002-2003 growing season. Each Partner translated the questionnaire into the language of their own country, making sure that all the nuances were translated correctly not only technically, but also in content, and then disseminated it to farmers in their own country.

In total, 1005 replies were obtained for analysis. The main survey produced 965 responses: 216 from Germany, 179 from Finland, 165 from Estonia, 154 from Poland, 136 from Sweden, and 115 from the UK; an additional 40 replies were obtained from pilot studies in Finland and in Estonia. Random postal surveys to OSR farmers (Finland, Estonia, UK partly) produced return rates from 25% (UK) to 46 % (Estonia), while targeted surveys provided higher returns: 61 % for Germany, 65 % for Sweden, and 'over 90%' for Poland. Different sampling methods had to be used, because the ideal method of random sampling of all rapeseed growers in each country was possible only in Finland and Estonia, where centralised data on growers are available. In other countries, regional and/or national advisory organisations or plant protection centres helped locate rapeseed growers, supplemented by targeted sampling of growers at farming events (UK, Finland, in part). We do not believe that the slight differences in the sampling methods between countries resulted in any inherent biases; in fact, analysis of the data showed that they are robust and reliable.

Here data concerning the important pest species, and methods and practices which farmers in the six EU-countries use to control them, are analysed and presented.

Results and discussion

Pest species requiring control measures

Europe-wide, only the pollen beetle was a problem requiring control in each country, in the growing season 2002-2003 (Table 1). The seed weevil, pod midge, and the cabbage stem flea beetle were the second most commonly mentioned as problems (important in 4 out of 6 countries), followed by the stem weevils (2-3 out of 6 countries). Six to seven different insect species were all very important in DE and PL, while in the UK 2-3 species and in SE, FI and EE only 1-2 species were problematic (Table 1).

Table 1. Frequency (in %) with which pest species were listed as requiring control measures during the growing season 2002-2003 in winter oilseed rape in the survey countries.

Pest species	Overall frequency (%)	DE	EE*	FI*	PL**	SE	UK
Meligethes aeneus	64.5	66	82	90	3	83	61
Psylliodes chrysocephala	20.3	43	0	0	2	15	63
Ceutorhynchus assimilis	17.0	56	2	1	28	4	11
C. napi	15.5	69	0	0	23	1	0
C. pallidactylus	14.0	47	0	0	22	0	14
Dasineura brassicae	11.5	25	0	1	22	10	11
Phyllotreta spp.	11.3	0	34	33	0	0	0
Delia radicum	2.0	12	0	0	0	0	0

* data refer to pests on spring/turnip rapeseed

** data for PL are not directly comparable with the others, because in PL each farmer listed only one species (the most important sp), while in other countries all spp requiring control measures were listed

These results are in general in good agreement with those of Bromand (1990), although it appears that in the UK, the pollen beetle is now ranked as more important, and the seed weevil and pod midge as less important than at the end of 1980s. In Germany, *C. napi* and *C. pallidactylus* also appear to have increased in importance.

Insecticide treatment patterns

The number of insecticide spray treatments were typically 2-4 per season in Poland (mean 2.6), 1-4 in Germany (1.8), 0-3 in the UK (1.4), and 0-3 in EE (1.3), SE (1.0), and FI (1.0). Paradoxically, the mean number of insecticide sprays was the same for conventional and IPM farmers (1.65 sprays/season), and in FI and PL farmers using IPM sprayed more often than conventional farmers. Also, the proportion of IPM farmers was highest in countries where the spraying rate also was highest (DE, PL, UK). Furthermore, overall, a higher proportion of IPM farmers used insecticide seed dressing than of conventional farmers (77.3% vs. 69.7%).

Insecticides in IPM

In all countries, the majority of farmers always sprayed all their OSR fields (81.0%), while overall, 14.7% sprayed some but not all fields, and a mere 4.3% reported having sprayed field edges only. Selective spraying was most common in the UK (30% of OSR farmers), Germany (27%), and Estonia (22%).

Out of nine different decision-making criteria for determining whether active pest control was necessary, the most frequent criterion mentioned by the farmers was simply seeing the pest on the crop (70.0% mentioned using), followed by using economic thresholds (47.3%), recommendation by crop protection consultant (24.0%), plant growth stage (16.3%), advisory service message (16.3%), advisory service general recommendation (8.7%), advice from neighbours (8.2%), commercial decision support system (2.7%), or spraying by the date (1.8%). Growers in PL used on the average 4.6 different criteria simultaneously to arrive at pest control decision, while in other countries clearly fewer criteria were sufficient: 2.7 in DE, 1.9 in SE and UK, and 1.6 in EE and FI. This pattern may be a direct reflection of the varying pest diversity and pest pressure in the surveyed countries, although it does not explain the difference between DE and PL, for example.

Background variables affecting insecticide use

A subset of the data (DE, FI, PL, UK) was used to study in greater detail the influence of some background variables on insecticide use frequency. Some interesting patterns emerged:

Spray frequency was 10% higher, when a farm worker or a contractor was in charge of insecticide treatments, rather than the farm owner him/herself.

Farm size had a significant impact: the bigger the arable area on the farm, the more frequently the OSR fields were sprayed. Taking spray frequency on small farms (arable area < 50 ha) as a reference, farms with 50-100 ha sprayed 12% more frequently, farms with 101-300 ha sprayed 13% more, and farms bigger than 300 ha sprayed 31 % more frequently their OSR fields than the small farms. It remains open to question whether this pattern reflects a higher pest pressure on bigger farms, or whether it relates to the attitudes of the farmer (or farm operator).

Quite interestingly, young farmers appeared to spray more frequently than older ones. When farmer age was < 35 years, spraying frequency was about 10% higher than that for older age groups. An explanation might be that young farmers are less experienced, and therefore spray more often 'for insurance' than older ones.

Rather surprisingly, the number of decision-making criteria used by farmers to decide whether or not to spray against insect pests, seemed to correlate with the frequency of spraying: the more you look, the more you will spray. Taking farmers using only one criterion as the baseline reference, those using 2 criteria sprayed 13% more often, those using 3 criteria sprayed 24% more often, and farmers using 4 or more criteria sprayed 31% more frequently.

Considering the basis for decision making as an explanatory variable for insecticide use patterns revealed that there are indeed differences, and that "how many times you spray depends on whom you listen to". The lowest number of insecticide treatments on OSR resulted when the farmer at least partly followed the advice of the extension services. Taking this as the baseline reference, other criteria increased the number of sprays as follows: inspecting the crop by oneself (see pest on crop) resulted in spraying 6% more often, following economic thresholds 11% more often, advice by consultant newsletter 13%, recommendation by crop inspectors 14%, relying on crop growth stage 15%, and using a decision support system (DSS) 23% more often. It is interesting to note that the largely publicly-funded extension service advice resulted in fewer sprayings in all the countries, compared with the advice from private sources such as crop consultants.

Acknowledgements

Support from the EU-project MASTER: MAnagement STrategies for European Rape pests (QLK5-CT-2001-01447) is gratefully acknowledged. However, without the cooperation of 1005 OSR growers as respondents to the survey, and the help of many federal and regional agricultural officers in the six survey countries, this study would not have been possible.

References

Bromand, B. 1990: Diversities in oilseed rape growing within the Western Palearctic Regional Section. – IOBC/wprs Bulletin 13 (4): 7-31.

Damage of registered Polish winter oilseed rape cultivars caused by pests

Marek Mrówczyński [1], Henryk Wachowiak [1], Czeslaw Musnicki [2], Michał Jodłowski [2] and Stefan Heimann [3]
[1] *Institute of Plant Protection, Miczurina 20, 60-318 Poznań, Poland*
[2] *Agricultural University, Mazowiecka 45/46, 60-623 Poznań, Poland*
[3] *Research Centre for Cultivar Testing, 63-022 Słupia Wielka, Poland*

Abstract: The aim of our investigations was to assess the degree of damage caused by pests to 42 cultivars of winter oilseed rape registered in Poland. The cultivars tested comprised 5 restored hybrids, 4 composite hybrids and 33 populations and work was carried out at Experimental Stations: Krościna Mała and Rarwino, at the plant breeding station Małyszyn. Large differences were observed in the degree of damage caused to the different cultivars by *Ceutorhynchus pleurostigma* Marsh., *Phorbia brassicae* Bche., *Ceutorhynchus napi* Gyll., *Ceutorhynchus quadridens* Panz., *Meligethes aeneus* F., *Dasyneura brassicae* Winn. and *Ceutorhynchus assimilis* Payk.

The endangerment of oilseed rape by pests in Poland

Felicyta Walczak and Marek Mrówczyński
Instytut Ochrony Roślin, ul. Miczurina 20, 60-318 Poznań, Poland

Abstract: Winter and spring oilseed rape crops are damaged by different pests throughout development. Crop losses can be reduced in the absence of control by about 20%, and in extreme cases can lead to partial or even total crop destruction. The pests reduce the quality and quantity the seed yield as well as making harvesting difficult, due to unevenness in ripening caused by plant compensation. Pests of economic importance include *Meligethes aeneus* L., *Ceutorhynchus nap*i Gyll., *Ceutorhynchus quadridens* Marsh., *Ceutorhynchus assimilis* Payk., *Dasyneura brassicae* Winn and occasional loss is attributed to *Phytomyza rufipes* Meig., Agrotinae, Thysanoptera, *Plutella cruciferarum* Zell. and also Gastropoda and birds. Endeavours to lower the cost and improve efficacy of crop protection methods depends mainly on: improvement monitoring to signal timing of interventions, qualification of thresholds of economic profitability of pest control, limitation of the number of executed interventions, using tank mixtures and correct choice of selective insecticides.

In this work the pests occurrence in winter and spring oilseed rape crops from 1999-2003 was studied across the different regions in Poland. Among the pests, 5 of them: *M. aeneus, C. napi, C. quadridens*, *C. assimilis* and *D. brassicae* caused most damage. These were summarized as a series of graphs and maps. The number of interventions registered to control pests in winter oilseed rape crops, the costs of spraying and insecticides as well as the proportional use of different groups of insecticides in Poland was also determined from historical records. Prior to the 1980s organophosphate preparations predominated. However from 1980 use of pyrethroids in prophylactic oilseed rape pest control increased. In the latter part of the 1990s the number of new biologically active substances registered for pest control in oilseed rape reduced. However, the number of generic insecticides is now increasing.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 99-101

The influence of glucosinolate content variability in the seeds and green matter of winter oilseed rape on the attack by selected pests

Eva Plachká, Oldřich Kolovrat and Viktor Vrbovský
OSEVA PRO Ltd., filial establishment of the Research Institute of Oilseed Crops at Opava, Purkyňova 10, 746 01 Opava, Czech Republic, opava@oseva.cz

Abstract: The glucosinolate content in the seeds of 30 cultivars of oilseed rape varied from 5.40 to 24.06 µmol/g dry matter. The glucosinolate content of cotyledons of three selected varieties (with seed dry maters from 9.73 to 12.4 µmol/g) varied from 9.30 to 12.14 µmol/g herbage dry matter. The changes in glucosinolate content were observed after mechanical injury to the leaves. Glucosinolate content varied on the third day after injury from 4.33 to 8.6 µmol/g herbage dry matter of intact leaves and from 23.22 to 24.19 µmol/g herbage dry matter of damaged leaves. The significant increase of indolyl glucosinolates was detected after leaf injury.

The relationship between seed glucosinolate content, emergence ability and size of plants to slug damage was observed using ten selected varieties oilseed rape. A scale with 6 degrees was used for the evaluation of slug injury: 1 = intact plant, 6 = whole plant or plant top is missing. The mean number of emerged plants varied from 22 to 31.5. Growth (size of plants) classification varied from degree1 (small plant) to degree 3 (big plant). Slug damage varied from 2.14 to 3.55 degrees. Data from our 1-year study period showed that glucosinolate content, emergence ability and size of plants influence slug damage.

Our research on the influence of glucosinolate content in oilseed rape seeds on slug damage was completed in 2004. The results obtained will be used for further research on the influence of glucosinolate content of the green plant matter on the incidence of the rapeseed sawfly (*Athalia rosae*) and stem weewils (*Ceutorhynchus napi* and *C. pallidactylus*).

Key words: Pests, glucosinolate, winter rapeseed

Materials and methods

The collection of c. 30 cultivars of double low winter oilseed rape registered in the Czech Republic was used as test material. Field plots (10 m^2) were established of each cultivar with two replicates of each cultivar. Assessments of the indidence of selected pests and associated damage was assessed at intervals throughout the growing season. The experiment was carried out over three seasons from 2001-2004.

The evaluation of slug damage of oilseed rape plants (laboratory test)

The organization and evaluation of tests were based on the instructions according to Plachká et al. (2003). Ten cultivars were evaluated. The laboratory tests were conducted using closed containers containing 33 plants (of identical cultivar). Each cultivar was replicated three times (i.e. using three containers). Each container was infested by three slugs when the plants had fully developed cotyledons (BBCH 10). Slug damage was evaluated every day for seven days after infestation. A scale with 6 degrees was used for the evaluation of slug injury:

1. Intact plant,
2. 1 – 25 % of leaf missing,
3. 26 – 50% of leaf missing,
4. 51 – 75% of leaf missing,

5. 76 – 90% of leaf missing, intact vegetative cone,
6. 91 – 100% of leaf missing inclusive of vegetative cone.

The evaluation of damage by Psylliodes chrysocephala and Phyllotreta spp in the field

We used the field evaluation based on EPPO methodics No. 1/73 (1997). A 2 x 2 m row of plants from each plot were evaluated. The damage was classified using the following scale:

1. Intact plant,
2. 1 – 10% of leaf missing,
3. 11 – 49% of leaf missing,
4. 50 – 100% of leaf is missing.

The evaluation of incidence of Ceutorhynchus pleurostigma in the field

The occurence of galls was evaluated on the roots of 25 plants sampled from each plot. The evaluation was conducted at two time points: (1) in autumn before freezing and (2) in spring at the beginning of stem elongation.

Analytical determination of glucosinolate content

The determination of glucosinolate content in seeds was done using a method developed in Research Institute of Oilseed Crops at Opava (Kolovrat, 1998). This method was based on the international standard (ISO, 1992). The determination of glucosinolate content in green matter is based on the published method by Hrnčiřík (1999) and the international standard (ISO, 1992).

The absolute values of the content of different glucosinolate groups and degrees of pest incidence were used for the evaluation of correlation (Microsoft Office 2000 - Excel, Rod et al., 1975).

Results and discussion

The evaluation of glucosinolate content

In the seeds (BBCH 00) the content of aliphatic glucosinolates was 54.7-91.2 %, aromatic glucosinolates was 0.4-15.8 % and indolyl glucosinolates was 5.5-35.1 %. In the cotyledons (BBCH 10) the content of aliphatic glucosinolates ranged between 2.9-5.7%, aromatic glucosinolates varied between 3.0-22.3 % and indolylglucosinolates from 37.4 to 82.8%. In the leaves (BBCH 13) the content of aliphatic glucosinolates was 0.7-44.2 %, aromatic glucosinolates was 5.6- 24.7 % and indolyl glucosinolates varied from 37.7 to 94.0 %.

In the oilseed rape seeds and leaves to developmental phase BBCH 13, the relative content of aliphatic glucosinolates decreased, the relative content of aromatic glucosinolates increased slowly and the relative content of indolyl glucosinolates increased. The content of aliphatic glucosinolates dominated in the seeds. The content of indolyl glucosinolates dominated in the leaves at developmental phase BBCH 10 and BBCH 13. In the roots, the content of indolyl glucosinolates dominated at the growth stage BBCH 10 and the content of aromatic glucosinolates dominated at the BBCH 13 growth stage.

The plant reaction to stress caused by mechanical damage of leaves was also observed. The content of indolyl glucosinolates after damage increased markedly to over 195% depending on plants cultivar and time after damage as compared with undamaged plants.

The evaluation of incidence of selected pests

Ceutorhynchus pleurostigma. The incidence of this pest was observed in field conditions. The conditions were unfavourable for this pest, so results can be evaluated from one year only. The relationship between glucosinolate content and attack of this pest was not detected.

Psylliodes chrysocephala and *Phyllotreta* spp. This evaluation was conducted over three years in field conditions. In the first season, we detected the dependence of pest incidence to

the total glucosinolate content and individual glucosinolate groups in plant cultivars. A statisticaly significant negative correlation occurred between pest incidence and indolyl glucosinolate content in the seeds and a positive correlation between the incidence of pests and content of indolyl glucosinolates in the cotyledons occurred. The correlation of the mean value of pest incidence from all three seasons to the content of indolyl glucosinolates in the seeds bordered on statistical significance (P = 0.05).

Slugs (*Deroceras* spp. and *Arion* spp.). This trial was conducted under laboratory conditions as described above. A negative statistically significant correlation was detected between slug incidence and content of aliphatic glucosinolates in the seeds.

Acknowledgements

This work was financially supported by the Ministry of Agriculture of the Czech Republic (Project No. QD 1350 and 1G 46061).

References

EPPO, 1997: Guidelines for the efficacy evaluation of insecticides: *Psylliodes chrysocephala*. PP 1/73(2). – Guidelines for the Efficacy Evaluation of Plant Protection Products. Vol 3. Insecticides & Acaricides, methodics No. 1/73(2). European and Mediterranean Plant Protection Organization.

Hrnčiřík, K. 1999: Významné produkty degradace glukosinolátů. – Disertační práce, VŠCHT, Praha. [The Significant Products of Glucosinolate Degradation. Dissertation Work, Chemical - Technological University at Prague.]

ISO 9167-1:1992(E): Rapeseed – Determination of Glucosinolates Content, Part 1: Method using high–performance liquid chromatography.

Kolovrat, O. 1998: Metodika stanovení obsahu glukosinolátů v semeni řepky metodou vysokoúčinné kapalinové chromatografie (HPLC). Závěrečná zpráva: Studium variability indolyglukosinolátů v semeni brukvovitých olejnin, č.projektu: EP 0960986043, č.programu: 02. – OSEVA PRO, s.r.o., o.z. VÚOl Opava. [Research Institute of Oilseed Crops Opava.]

Plachká, E., Kurowská M., Ráčil, K. 2003: Metodika k hodnocení biologické účinnosti moluskocidů a jiných zoocidů a zoocidních (insekticidních) mořidel proti slimákům a plzákům v řepce olejce a jiným brukvovitým plodinám (umělá infestace). Metodický návod. Závěrečná zpráva: Vývoj komplexních systémů ochrany proti významným chorobám a škůdcům obilnin, řepky a hrachu s cílem širokého uplatnění integrovaných způsobů ochrany, č.projektu QD1350. – OSEVA PRO s.r.o., o.z. VÚOL Opava.

Rod, J. & Vondráček, J. 1975: Polní pokusnictví – Pokusnická technika se základy biometriky. Státní pedagogické nakladatelství, n.p. Praha. – Vydání 1/1.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 103-107

The damage of seeds and seedlings of winter oilseed rape cultivars caused by *Deroceras reticulatum* (Müller) (Gastropoda: Pulmonata: Agriolimacidae) and *Arion lusitanicus* Mabille (Gastropoda: Pulmonata: Arionidae)

Tomasz Kałuski and Jan Kozłowski
Institute of Plant Protection, Department of Zoology, Miczurina 20, 60 – 318 Poznań, Poland

Abstract: In laboratory conditions the susceptibility of 18 oilseed rape cultivars to damage caused by *Deroceras reticulatum* and *Arion lusitanicus* was estimated. For each slug species three experiments were carried out: one with seeds, one with seedlings at growth stage 08 – 10 BBCH and one with seedlings at growth stage 10 – 11 BBCH. Significant differences in damage to seeds and seedlings caused by *Deroceras reticulatum* and *Arion lusitanicus* were observed, but only on some days of the test. The most severely damaged cultivar, damaged by both slug species, was Lisek, and the least damaged cultivars were Bazyl, Extreme and Californium.

Key words: *Deroceras reticulatum, Arion lusitanicus*, oilseed rape, slugs, cultivars

Introduction

Oilseed rape (*Brassica napus* ssp. *oleifera*) is one of the main European oil crops (Weiss, 1983; Kimber & McGregor, 1995). Oilseed rape oil is used either in human consumption or in industry as lubricants, detergents and soaps. The European Community in the 1980's recommended that the concentrations of glucosinolates in oilseed rape should be reduced (Moens & Glen, 2002). From the 1970's the economic importance of slugs has increased, as a result of reduce tillage (Voss *et al.*, 1998). During the last ten years in Poland, slugs, mainly *Deroceras reticulatum* and *Arion lusitanicus*, were very serious pests in oilseed rape crops (Kozłowski 1999, 2002; Kozłowski & Kozłowska 2002). Plants are most at risk at the seedling stage (growth stage 07 – 10 BBCH) when slugs feeding immediately after plant emergence kill and reduce plant stands. Damage in oilseed rape has increased with the introduction of low glucosinolate cultivars (Glen *et al*., 1990; Moens *et al.,* 1992, Byrne & Jones, 1996). Some data in the literature show that plant cultivar may be a very important factor in determining damage severity caused by pests. The aim of the presented studies was to determine the palatability of 18 low-glucosinolate winter oilseed rape cultivars to *D. reticulatum* and *A. lusitanicus* slugs.

Materials and methods

Experiments were carried out on plants of 18 oilseed rape cultivars (Table 1 and 2) with slugs *Deroceras reticulatum* and *Arion lusitanicus*. Adult slugs were field-collected a few weeks before experiments began, and kept in laboratory conditions in clear plastic containers (19°C ± 2°C, RH 95% ± 3%, natural light). The containers were filled with 5 cm loam and humus soil. Slugs were fed with wheat and oilseed rape seeds, carrots, potatoes, wheat bran with calcium carbonate and Chinese cabbage leaves. The experiments were carried out in laboratory conditions at 17°C day and 14°C night, 12 h day and RH 95% ± 3%. The multiple-

choice tests for oilseed rape seeds (03 – 07 BBCH), seedlings soon after germination (08 – 10 BBCH) and for seedlings with 2 cotyledons (10 – 11 BBCH) were made separately. These tests were carried out in a nested block design for 18 cultivars with 6 replicates, separately for *D. reticulatum* and *A. lusitanicus*.

The experiments with winter oilseed rape seeds were carried out in 3 plastic containers (54.5 x 76.0 x 13.5 cm) with ventilation holes, randomly divided into 36 small plots. Five oilseed rape seeds were placed in Petri dishes (70 mm diameter), lined with damp filter paper. For each cultivar, six identical dishes were prepared. Two open dishes with seeds of each cultivar were placed into every container. Ten *D. reticulatum* or *A. lusitanicus*, starved previously for 24 h, were placed in each box.

The tests with seedlings of oilseed rape were carried out in plastic boxes (80 x 40 x 20 cm) with a 4.5 cm soil layer, the surface of which was divided into 36 small plots. On randomly chosen plots, seeds of 18 oilseed rape cultivars were sown, five seeds of each cultivar on two plots. Ten *D. reticulatum* or *A.* lusitanicus slugs, starved for 24 h before the experiment, were placed in each box. However in experiments with seedlings soon after germination (08 – 10 BBCH) slugs were placed in boxes 24 hours after the seeds were sown, and in experiments with seedlings in the 2 cotyledons phase (10 – 11 BBCH), slugs were introduced when the seeds had germinated.

In all six experiments the percentage of damaged seeds or seedlings was noted daily. The observations were carried out for a few days according to the feeding rate. In all tests, visual evaluation (using a 5 – degree scale: no damage, 25%, 50%, 75%, 100% of plant area consumed) of seedlings or seed damage was carried out. From these observations, for each experimental unit, a weighted mean percentage of the damaged surface of rape seeds or seedlings was calculated. For each of six experiments variance analysis and Tukey's test at $\alpha = 0.05$ using a nested block design were made.

Results and discussion

The damage to seeds and seedlings of oilseed rape by Deroceras reticulatum

The oilseed rape seeds did not show statistically significant differences between damage to particular cultivars. However the most severe damage to the cultivars: Batory (30% of surface of seeds damage on the third day of slug feeding), Kaszub (43.3%), Lisek (36.7%), Californium (26.7%) and Extreme (25%) were observed (Table 1). The least damaged seeds were of Liclassic (from 1.7% in the first day to 13.3% in the third day of slug feeding), Rafaela (1.7% – 15%) and Bazyl (3.3% - 16.7%) cultivars (Table 1).

In experiments with oilseed rape seedlings in growth stage 08 – 10 BBCH, statistical differences ($p=0.008$) in damage to particular cultivars were observed 5 days after slug feeding behan, but the Tukey's test did not differentiate the cultivars. From the third to the fifth day of slug feeding the most serious damage to cultivars: Rasmus (65% – 86.7% of damaged plant surface), Libomir (40% – 86.7%), Digger (60% – 83.3), Bazyl (47.5% – 83.3%) and Rafaela (43.3% – 83.3%) were noted (Table 1). After five days of slug feeding the least damaged cultivars in this growth phase were the Kaszub (53.3%) and Extreme (46.7%) (Table 1).

The experiment with seedlings in growth stage 10 – 11 BBCH showed significant differences after the third and fifth day of slug feeding (Table 1). After three days of slug feeding, the percentage of seedling damage to the Batory (72.5%), Bazyl (62.5%), Contact (85%), Lisek (88.3%) and Cazek (87.5%) cultivars were statistically higher than the percent damage to seedlings of Extreme (36.7%). On the fifth day of grey field slug feeding the cultivars: Batory (87.5%), Bazyl and Digger (82.5%), Cazek and Contact (92.5%), Liclassic

and Lisek (83.3%) and Rasmus (85.8%) were significantly more severely damaged than seedlings of the Extreme (55%) and Kana (77.5%) cultivars. After seven days of observations the most seriously damaged seedlings were of Contact (96.7%), Cazek (95.8%), Liclassic and Lisek (95%) (Table 1).

Table 1. The percentage of damaged seeds and seedlings of different winter oilseed rape cultivars caused by *D. reticulatum* in multiple-choice test and results of Tukey's test at α=0.05 (values within each column followed by the same letter are not significantly different)

	Seeds 03-07 BBCH			Seedlings 08-10 BBCH			Seedlings 10-11 BBCH			
Days:	1	2	3	3	4	5	1	3	5	7
p =	0.467	0.415	0.656	0.187	0.092	0.008	0.580	0.003	0.000	0.099
Batory	5	8.3	30	56.7	69.2	80 a	34.2	72.5 a	87.5 a	94.2
Bazyl	3.3	5	16.7	47.5	70.8	83.3 a	27.5	62.5 a	82.5 a	90.8
Californium	1.7	8.3	26.7	42.5	65.8	72.5 a	20.8	55.0 ab	68.3 ab	89.2
Cazek	0	3.3	23.3	45.8	65	78.3 a	49.2	87.5 a	92.5 a	95.8
Contact	0	6.7	26.7	66.7	73.3	79.2 a	43.3	85.0 a	92.5 a	96.7
Digger	0	0	21.7	60	73.3	83.3 a	29.2	70.0 ab	82.5 a	92.5
Extreme	8.3	13.3	25	36.7	42.5	46.7 a	15.8	36.7 b	55.0 b	87.5
Kana	0	10	25	38.3	50.8	60 a	28.3	51.7 ab	77.5 b	92.5
Kaszub	3.3	20	43.3	26.7	44.2	53.3 a	27.5	65.0 ab	75.0 ab	90.8
Libomir	0	11.7	23.3	40	67.5	86.7 a	26.7	47.5 ab	68.3 ab	90.0
Liclassic	1.7	5	13.3	46.7	66.7	79.2 a	30.0	75.0 ab	88.3 a	95.0
Lirajet	0	5	28.3	45.8	69.2	84.2 a	28.3	60.8 ab	70.0 ab	89.2
Lisek	3.3	8.3	36.7	50	70.8	75.8 a	49.2	85.0 a	88.3 a	95.0
Mazur	1.7	5	26.7	25.8	47.5	58.3 a	18.3	63.3 ab	79.2 ab	88.3
Olpop	8.3	21.7	40	62.50	76.7	80.8 a	24.2	67.5 ab	82.5 ab	90.8
Pomorzanin	0	8.3	23.3	26.7	46.7	61.7 a	24.2	56.7 ab	66.7 ab	87.5
Rafaela	1.7	3.3	15	43.3	67.5	83.3 a	25.8	61.7 ab	78.3 ab	92.5
Rasmus	0	1.7	26.7	65	75	86.7 a	33.3	80.8 ab	85.8 a	94.2

The damage to seeds and seedlings of oilseed rape by Arion lusitanicus

The observations of damage to oilseed rape seeds caused by *Arion lusitanicus* showed statistical differences only in the third day of slug feeding, but the Tukey's test did not differentiate particular cultivars (Table 2). The most severe damage on the third day of the experiment was observed to cultivars Rasmus and Olpop (73.3%), Mazur (70%) and Lisek (63.3%). The least damaged were seeds of Batory (16.7%) and Kana (23.3%) cultivars (Table 2).

In the experiment with seedlings in growth stage 08 – 10 BBCH, no significant differences were noted. After three days of *A. lusitanicus* feeding, most plants were completely eaten. However, the most severely damaged cultivars were Kaszub (96.7% of plant surface eaten in the fourth day of observations), Extreme (92.5%), Olpop (88.3%), Kana and Californium (86.7%) (Table 2).

The observations in tests with seedlings in growth stage 10 – 11 BBCH showed statistically significant differences between particular cultivars on the third and fifth day of slug feeding. On the third day of the experiment, cultivars: Lisek (91.7%), Liclassic (88.3%), Contact (87.5%) and Rasmus (84.2%) were significantly more severely damaged than the Extreme (49.2%) cultivar (Tab. 2). Though variance analysis on the fifth day of the experiment showed statistically significant differences (p=0.000) the Tukey's test did not differentiate the cultivars. After seven days of slug feeding, some cultivars were completely destroyed: Bazyl, Contact, Digger, Liclassic, Lirajet and Rasmus (100% of plant surface eaten), and the remaining cultivars were damaged at almost 100% (from 96.7% to 99.17% of plant surface eaten) (Table 2).

Table 2. The percentage of damaged seeds and seedlings of different winter oilseed rape cultivars caused by *A. lusitanicus* in multiple choice test and results of Tukey's test at α=0.05 (values within each column followed by the same letter are not significantly different)

	Seeds 03-07 BBCH			Seedlings 08-10 BBCH		Seedlings 10-11 BBCH			
Days:	1	2	3	3	4	1	3	5	7
p =	0.394	0.443	0.048	0.215	0.236	0.520	0.004	0.000	0.698
Batory	3.3	6.7	16.7 a	70	89.17	40	75 ab	92.5 a	99.2
Bazyl	3.3	10	33.3 a	53.3	76.67	40.8	72.5 ab	98.3 a	100
Californium	0	16.7	30 a	63.3	86.67	25.8	70.8 ab	93.3 a	99.2
Cazek	6.7	13.3	26.7 a	50	70	30.8	80.8 ab	93.3 a	96.7
Contact	0	6.7	50 a	46.7	76.67	40	87.5 a	99.2 a	100
Digger	0	33.3	60 a	61.7	73.33	30	72.5 ab	96.7 a	100
Extreme	0	10.0	33.3 a	70	92.50	27.5	49.2 b	88.3 a	98.3
Kana	0	0	23.3 a	70	86.67	35.8	60.8 ab	94.2 a	99.2
Kaszub	0	13.3	50 a	70	96.67	42.5	73.3 ab	93.3 a	97.5
Libomir	3.3	23.3	53.3 a	46.7	80	33.3	70.8 ab	94.2 a	99.2
Liclassic	0	0.0	23.3 a	36.7	66.67	50	88.3 a	98.3 a	100
Lirajet	3.3	13.3	40 a	20	70	35.8	71.7 ab	92.5 a	100
Lisek	0	13.3	63.3 a	76.7	80	63.3	91.7 a	96.7 a	99.2
Mazur	0	13.3	70 a	66.7	76.67	52.5	73.3 ab	91.7 a	98.3
Olpop	0	0	73.3 a	56.7	88.33	43.3	74.2 ab	89.2 a	99.2
Pomorzanin	0	13.3	40 a	60	90	42.5	67.5 ab	92.5 a	99.2
Rafaela	0	26.7	60 a	40	77.50	25.8	72.5 ab	94.2 a	98.3
Rasmus	0	10	73.3 a	60	95	45.8	84.2 a	94.2 a	100

Significant differences in damage of seeds and seedlings caused by *Deroceras reticulatum* and *Arion lusitanicus* were observed only in some days of slug feeding. This is in contradiction to previous research (Kozłowski & Kałuski, 2004) where cultivars with low and high glucosinolate contents were examined. The source of the general lack of significant

differences between cultivars is probably the low level of glucosinolates in all modern oilseed rape cultivars. However the most severely damaged material by *D. reticulatum* and *A. lusitanicus* were seeds of Californium, Lisek and Libomir cultivars, and seedlings of Contact, Lisek, Liclassic and Rasmus cultivars. Farmers should therefore not use these cultivars on areas where slug damage to plants has been previously noted.

References

Byrne, J. & Jones, P. 1996: Responses to glucosinolate content in oilseed rape varietes by crops pest (*Deroceras reticulatum*) and non-pest slug species (*Limax pseudoflavus*). – Ann. Appl. Biol. 128 (Suppl. Tests Agrochem. Cultivars 17): 78-79.

Glen, D.M., Jones, H., Fieldsen, J.K. 1990: Damage to oilseed rape seedlings by the field slug *Deroceras reticulatum* in relation to glucosinolate concentration. – Ann. Appl. Biol. 117: 197-207.

Kimber, D.S. & McGregor, D.I. 1995: The species and their origin, cultivation and world production. – In: Brassica Oilseed, Production and Utilization. CAB International, Wallingford: 1-7.

Kozłowski, J. & Kałuski, T. 2004: Susceptibility of winter oilseed rape cultivars to damages caused by *Arion lusitanicus* Mabille (Gastropoda: Pulmonata: Arionidae). – Rośliny Oleiste 25: 177-186.

Kozłowski, J. & Kozłowska, M. 2002: Assessment of plant damages and intensity of *Deroceras reticulatum* (Müller) occurrence in winter oilseed rape and winter wheat. – J. Plant Protection Res. 42(3): 229-237.

Kozłowski, J. 1999: Ślimaki (*Gastropoda: Stylommatophora*) – niedoceniane szkodniki roślin uprawnych w Polsce. – Post. Nauk Roln. 6: 39-50.

Kozłowski, J. 2002: Ochrona rzepaku i zbóż przed ślimakami. – Institute of Plant Protection, Poznań: 1-20.

Moens, R. & Glen, D.M. 2002: Agriolimacidae, Arionidae and Milacidae as pests in West European oilseed rape. – In: Molluscs as Crop Pests. CAB International, Wallingford: 425-439.

Moens, R., Couvreur, R., Cors, F. 1992: Influence de la teneur en glucosinolates des variétés de colza d'hiver sur les dégats de limaces. – Bull. Rech. Agr. Gemb. 27: 289-307.

Voss, M.C., Ulber, B., Hoppe H.-H. 1998: Impact of reduced and zero tillage on activity and abundance of slugs in winter oilseed rape. – Z. PflKrankh. PflSchutz 105(6): 632-640.

Weiss, E.A. 1983: Oilseed Crops. – Longman, Harlow, UK.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 109-114

Study of harmful Anthomyiidae in oilseed rape fields with different drilling dates

Wolfgang Büchs and Sabine Prescher
BBA, Institute for Plant Protection in Field Crops & Grassland, Messeweg 11/12, D-38108, Braunschweig, Germany, w.buechs@bba.de

Abstract: Over the past couple of years in Germany, the cabbage root fly (*Delia* spp.) has caused increasing damage in winter oilseed rape crops, particularly by the feeding of larvae on the roots of young plants in autumn. The intensity of infestation of the oilseed rape by root flies is a function of the development of the plant. In fields with different drilling dates, the Anthomyiidae spectrum was assessed by emergence traps. Overall, 7 Anthomyiidae species with the potential to feed on oilseed rape were recorded, four of them were abundant. It was shown that drilling date heavily influences the species assemblage of pest Anthomyiidae and their dominance. In early-drilled winter oilseed rape *Delia radicum* was dominant, while in later drilled winter oilseed rape fields *Delia platura* predominated. Other species like *Delia florilega* and *Botanophila fugax* were also more abundant in later-drilled oilseed rape fields. Thus, the change of Anthomyiidae assemblages and their dominance position according to drilling date can influence the success of control measures.

Key words: *Delia* spp., *Botanophila fugax*, Anthomyiidae, cabbage root fly, oilseed rape, drilling dates, crop management

Introduction

The cabbage root fly (*Delia* spp.) has caused increasing damage in winter oilseed rape crops in Germany over the past few years, particularly by feeding of larvae on the roots of young plants in autumn. Larval feeding damage causes crevices in the roots and in the worst cases the roots are severed completely. Incidences of severe damage were first reported from the northern and eastern parts of Germany (Mecklenburg-Vorpommern, Schleswig-Holstein, Brandenburg, Sachsen-Anhalt, Thüringen). Occasional damage has since been reported from nearly all other parts of Germany. A similar development in the UK of increasing damage by *Delia* root flies was reported in the mid 1980's by Skinner & Finch (1987). Today, in Germany there are approaches available to control cabbage root fly by special seed dressings. However, this article shows that severe damage can be avoided by very simple cultural measures (crop management). The oilseed rape growing area in Germany has increased from ~60,000 ha in 1986 to ~ 1.3 million ha today, thus the density of oilseed rape fields in the landscape is partly responsible for the spread of cabbage root flies and their new status as pests. Another factor important in this issue is that management practice has changed to increasingly early drilling of winter oilseed rape. Fifteen years ago in most regions of Germany, oilseed rape was drilled at the end of August or locally, even at the beginning of September; today drilling occurs mostly in mid August, with some local tendencies to drill within the first ten days of August. Thus, when the third generation of *Deila radicum* is about to lay eggs, it finds well developed oilseed rape plants which offer all the features cabbage root flies obviously need for oviposition and development.

The species of genus *Delia* which is said to be mainly responsible for the damage in oilseed rape is *Delia radicum*. Hennig (1976) mentions 3-4 generations of this species in

central Europe which are not sharply separated. Imagines of these generations occur at the beginning of May until the end of June, at the beginning of July until the end of August and mid August until the end of October. Within the first generation, there are early and late phenotypes, the latter having an additional diapause resulting in delayed emergence in spring. This enables *D. radicum* to optimize the use of available resources (Biron *et al*., 1998). Eggs are laid in the soil, which consists in optimum cases of particles of about 1 mm in size (Traynier, 1967a, b). The egg-laying is combined with a certain search-flight behaviour. For egg-laying the optical composition and structure of the place of landing is important in addition to olfactory cues for example ingredients of „mustard oil" (linol- and linolenacids, eruca acids and isothiocyanates) (Traynier, 1967a, b; Zohren,1968).

Materials and methods

Anthomyiidae flies were caught by emergence traps (diameter 60 cm) in two locations: Eickhorst (about 10 km north of Braunschweig) and Vogelsang (about 30 km southeast of Göttingen). The trapping period in Vogelsang was 21.03.- 29.06.2005, and 06.04-29.06. 2005 in Eickhorst. In each field, four emergence traps were installed and controlled weekly.

In both locations, two fields were compared with two different drilling dates and different performance in emergence of the crop. The drilling dates differed by 6 days in Vogelsang and 12 days in Eickhorst. The soil type was similar in both fields in Vogelsang, however it differed in Eickhorst (Table 1).

Table 1. Description of study sites with different drilling dates.

Location	**Vogelsang**	**Vogelsang**	**Eickhorst**	**Eickhorst**
Drilling date	7.8.04 **germination early**	13.8.04 **germination delayed**	22.8.04 **germination early**	3.9.04 **germination delayed**
Soil	clay	clay	clay	sandy loam
Number of emergence traps	4	4	4	4

Results and discussion

Economically-relevant damage is assumed to occur when the two highest stages of damage from a 6-point assessment criteria are achieved (Erichsen et al., 2004).

Usually, only *Delia radicum* is mentioned as pest of oilseed rape. However, through use of emergence traps in oilseed rape fields, seven species of Anthomyiidae were recorded. Of these, the larvae of five are damaging to oilseed rape although some have other hosts (Table 2). Beside those mentioned, we also found in oilseed rape *Delia antiqua* and *Delia coarctata*, however, at least the latter is well known as pest of cereals and will not harm oilseed rape.

Table 2. Species of Anthomyiidae (Diptera: Brachycera) with larvae that are harmful to oilseed rape

Species	Common name of larvae	Economically important pests of (beside oilseed rape):
Delia radicum (Linnaeus, 1758)	Cabbage root fly	Cauliflower, savoy cabbage, brussel sprouts, radish
Delia floralis (Fallen, 1824)	Turnip root fly	Radish
Delia platura (Meigen, 1824)	Seed corn maggot	Shrub beans, runner beans, peas, cauliflower, maize
Delia florilega (Rob.-Des., 1830)	Bean seed maggot	Lupin, maize, asparagus, beans
Botanophila fugax (Meigen, 1826)		Cauliflower, savoy cabbage, Brussels sprouts

The Anthomyiidae species recorded in oilseed rape fields show significant differences in their biology, i.e. their phenology, occurrence in oilseed rape fields, number of generations, location of egg laying, time and location of pupation and food preferences or host plant spectrum (Table 3). These considerable differences of the *Delia* species biology can have significant effects on assessment and control of these pest populations. These differences cause the need for different and sometimes complex approaches to control these flies, since control measures which are designed for one of the species (mostly *Delia radicum*) do not function well when other species dominate. For example *Delia radicum* and *Delia antiqua* show some similarities: both species occur as adult flies at the end of April to the beginning of May and have up to 3 generations per year. Both species lay about 10 eggs/plant and the larvae hatch after 3-8 days. However, differences include:

- larval development, for which *Delia radicum* needs longer (~1 week during the vegetative period) than *Delia antiqua*
- Pupation: *Delia antiqua* pupates deeper in the soil than *Delia radicum* (pupation usually occurs at 20 cm and in the upper 5 cm, respectively)

Table 3. Differences in the biology of Anthomyiidae species emerging in oilseed rape

Species	number of generations	Duration of larval development (days)	depth of pupation in the soil (cm)
Delia radicum	3	21-28	upper 5 cm
Delia antiqua	2-3	15-20	10-35 cm
Delia floralis	1	15-20	10-35 cm
Delia platura	up to 5	21	no data available
Delia florilega	up to 5	21	no data available

The biology of *Delia platura* however is completely different to that of *Delia radicum*: *Delia platura* adults can hatch as soon as March and can have up to four generations per year. Consequently, duration of development is much shorter than for *Delia radicum*. Furthermore,

this species is attracted by organic substances and is able to lay its eggs into the bare field soil, before drilling of the oilseed rape crop. The first larval stages are able to survive by feeding from organic substances until emergence of the crop plants.

Table 4. Abundances (individuals/m²) and dominance (%) of Anthomyiidae caught in emergence traps from two fields with different drilling dates at two different locations in Germany

Location	**Vogelsang**		**Vogelsang**		**Eickhorst**		**Eickhorst**	
Drilling date	7.8.04 **germination early**		13.8.04 **germination delayed**		22.8.04 **germination early**		3.9.04 **germination delayed**	
	ind/m²	%	ind/m²	%	ind/m²	%	ind/m²	%
Delia florilega males	1	1.2	2	2.4	0	0.0	**12**	**6.4**
Delia platura males	7	8.4	**16**	**19.5**	1	1.2	**27**	**13.8**
Delia platura / florilega females	15	18.1	**40**	**48.8**	13	15.1	**86**	**43.9**
Delia radicum	**57**	**68.7**	21	25.6	**32**	**37.2**	7	3.6
Botanophila fugax	-	-	-	-	39	**45.3**	**63**	32.1

If the hatching rates of Anthomyiidae flies in the earlier and later sown oilseed rape fields are compared, remarkable differences in the abundance of species are seen (Table 4). For *D. radicum* in both locations about 2.5-times more flies were recorded in early-sown than late-sown oilseed rape. Conversely, *D. platura* showed 3.5-5.0 times higher hatching rates in later-drilled oilseed rape fields. A similar effect was observed for *Botanofila fugax*, however this species was recorded only in one location (Eickhorst). The drilling date has a significant influence on which species is dominating and which pest is potentially the main threat to the crop. In early-sown oilseed rape, *Delia radicum* always dominates; in later-drilled crops *Delia platura* and other species predominated (Table 3). Other species like *Delia florilega* and *Botanophila fugax* were also more abundant in later-drilled oilseed rape crops, but at least for *Botanophila fugax* this was not reflected in dominance (Table 4). Thus, the choice of drilling date determines the structure of Anthomyiidae assemblages and the dominance position of the species. Consequently, it can influence heavily crop damage and the success of control measures. Overall, the intensity of infestation of the oilseed rape by root flies is a function of the development of the crop plant.

Table 5 shows that severe damage to the oilseed rape plants could be only recorded when oilseed rape was drilled early. This damage is obviously caused primarily by *Delia radicum* according to the dominance of this species in early drilled oilseed rape fields. This shows however, that already by a delay of drilling for only a couple of days an economical damage of the crop plant can avoided. Also, at later drilling dates *Delia radicum* is still present in remarkable numbers but its damage is less, possibly because of the competition with other, less harmful Anthomyiidae species.

Table 5. Damage of oilseed rape plants by Anthomyiidae species in early and late drilled oilseed rape fields. Damage (%) = percentage of damaged plants; Average value of estimated damage was made using the 6 degree scale of Erichsen *et al*. (2004)

Location	**Vogelsang**	**Vogelsang**	**Eickhorst**	**Eickhorst**
Drilling date	7.8.04 **germination early**	13.8.04 **germination delayed**	22.8.04 **germination early**	3.9.04 **germination delayed**
Average value of estimated damage	**3.4**	2.0	**2.7**	1.3
Damage (%)	**31.4**	11.0	**57.5**	15.0

Conclusions

In oilseed rape crops with different sowing dates at two locations in Germany, up to 7 Anthomyiidae species occurred in different combinations and with different species dominance. Drilling date can affect the degree of damage to the crop plant and the success of control measures. Considerable damage to oilseed rape plants can be caused by *Delia radicum* which prefers well-developed oilseed rape plants in autumn. Therefore, this Anthomyiidae-species is a potential threat to oilseed rape particularly when the crop is drilled early (in Germany ~20 August and before). In oilseed rape crops that are drilled later, damage is reduced because *Delia radicum* has to compete with other species (with lower damage potential).

It was shown that a delay of the drilling date of only 4 days has the potential to significantly reduce the damage of these fly larvae to the oilseed rape roots. Further measures which may reduce the attack by *Delia radicum* and other *Delia*-species are:

- the use of hybrid cultivars, which seem to be less susceptible against *Delia*-attack
- thorough tillage measures to reduce stubble size after harvest of oilseed rape
- use of volunteer oilseed rape as trap crop for *Delia*-species with thorough subsequent tillage measures in order to bury them deep into the soil immediately after egg-laying of cabbage root flies of the second generation
- intensive tillage measures like ploughing, and thorough preparation of the seed bed after harvesting oilseed rape
- reduction of seed density
- delayed control of weeds

For further details regarding biological control of cabbage root flies in Canola, a spring crop, see also Dosdall *et al*. (2003).

Acknowledgements

We cordially thank Fabian Zelmanski for technical support in the field and laboratory and the UFOP (Union für die Förderung von Öl- und Proteinpflanzen) for financial support (project-no. 521/052)

References

Biron, D., Langlet, X., Boivin, G. & Brunel, E. 1998: Expression of early and late-emerging phenotypes in both diapausing and non-diapausing *Delia radicum* L. pupae. – Entomologia Experimentalis et Applicata 87: 119-124.

Dosdall, L.M., Clayton, G.W., Harker, K.N. & O´Donovan, J.T. 2003: Insect – agronomic interactions in canola integrated pest management. – 11th International Rapeseed Congress, 6-10th July 2003, Kopenhagen, Denmark 4: 1051-1053.

Erichsen, E., Hünmörder, H. & Kruspe, C. 2004: Die Kohlfliege – Hauptschädling auf den Winterrapsflächen im Herbst 2003. – Raps 22 (1/2004): 4-9.

Hennig, W. 1976: Anthomyiidae. – In: Lindner, E. (ed.): Die Fliegen der paläarktischen Region, Part 63a: 681-974, Stuttgart.

Skinner, G. & Finch, S. 1987: Oilseed rape crops as a source of cabbage root fly infestations for cruciferous vegetable crops. – IOBC/wprs Bulletin 10(4): 61-66.

Traynier, R.M.M. 1967a: Effect of host plant odour on the behaviour of the adult cabbage root fly, *Erioischia brassicae*. – Entomologia Experimentalis et Applicata 10: 321-328.

Traynier, R.M.M. 1967b: Stimulation of oviposition by the cabbage root fly, *Eroischia brassicae*. – Entomologia Experimentalis et Applicata 10: 401-412.

Zohren, E. 1968: Laboruntersuchungen zur Massenzucht, Lebensweise und Eiablage und Eiablageverhalten der Kohlfliege *Chortophila brassicae* Boúche. – Zeitschrift für angewandte Entomologie 62: 139-188.

Pest damage to oilseeds – Solutions (i)

Insecticides and issues regarding insecticide resistance

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 117-123

Efficacy of *Trichogramma chilonis* (Ishii) and some new synthetic insecticides against *Helicoverpa armigera* (Hübner) in sunflower

Waqas Wakil[1], Mansoor-ul-Hasan[2], Shahbaz Talib Sahi[3] and Y.J. Kwon[4]
[1] *Department of Entomology, University of Arid Agriculture, Rawalpindi, Pakistan, e-mail: waqaswakeel@hotmail.com*
[2] *Department of Agricultural Entomology, University of Agriculture, Faisalabad, Pakistan*
[3] *Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan*
[4] *School of Applied Biology and Chemistry, Kyungpook National University, Daegu 702-701, Korea*

Abstract: The present studies were conducted to assess the effectiveness of the parasitoid *Trichogramma chilonis* against the pest *Helicoverpa armigera* and to examine the insecticidal effects of some new insecticides against *H. armigera*. Four new synthetic insecticides: Lannate 40 SP, Proclaim 019 EC, Steward 150 SC and Tracer 240 SC were used and the eggs of *T. chilonis* were released at 60,000, 80,000 and 1,00,000 acre^{-1}. All the treatments controlled the pest but among the insecticides, Steward proved to be the most effective in reducing the larval population. *Trichogramma chilonis* suppressed the larval population of the pest and this effect was more pronounced with increased numbers of eggs released in the field. Maximum decrease in larval population was observed when the eggs of the parasitoid were released at 1,00,000 acre^{-1}. It was concluded that the parasitoids might become an integral part of management package only by augmentation with new insecticides. But at the same time, to avoid resistance in the insect pests, conventional insecticides must be replaced with the newly emerging insecticides, which are safer.

Key words: Efficacy, *Helicoverpa armigera*, *Trichogramma chilonis*, insecticides, sunflower

Introduction

The sunflower headworm (*Helicoverpa armigera* Hübner) is a versatile polyphagous agricultural pest. Host species for *H. armigera* come from a broad range of plant families and include important agricultural crops such as cotton, maize, chickpea, pigeonpea, sorghum, sunflower, soybean and groundnuts (Fitt, 1989). Biologically, it is one of the most successful pests due to its high fecundity, high migration potential, wide host range, and diapausing behavior to overcome unfavorable environmental conditions. In sunflower, the larvae feed on the florets and developing seeds. While feeding it thrusts it head inside the sunflower head leaving the rest of its body outside (Amjad *et al.*, 2005). It is considered a "difficult to control" pest because of its ability to develop resistance against almost all the conventional insecticide chemistries applied for its control (Ahmad *et al.*, 2001). The *Helicoverpa* problem is therefore serious and creates a need for the development of management strategies that are less dependent on chemical insecticides and/or less conducive to the development of resistance to current conventional chemical control measures.

Natural enemies may reduce populations of *H. armigera* (King and Coleman, 1989). During the last two decades, considerable work has been carried out on the use of parasitoids (Nagarkatti, 1982) and predators (Greathead and Girling, 1982; King *et al.*, 1982). Parasitoids might become an integral part of management package if augmented with insecticides. In Pakistan, *H. armigera* resistance to endosulfan, pyrethroids and organophosphates has already

been reported (Ahmad *et al.*, 1995, 1997, 1998a,b, 1999). To avoid resistance in the insect pests, conventional insecticides need to be replaced with the newly emerging insecticides, which are safer and more effective.

We assessed the effectiveness of dispersal of *T. chilonis* eggs in control of *H. armigera*, and examined the insecticidal effect of some new synthetic insecticides against *H. armigera* on sunflower.

Materials and methods

Field trial and crop management

The experiment was conducted using sunflower crops during 2005 at the research farm of University of Arid Agriculture, Rawalpindi, Pakistan. All the cultural practices including manual weeding, fertilizer applications, irrigation and soil cultivation were performed according to usual practices. Plants were spaced 23 cm apart along single rows spaced at 75 cm.

Trial design

The experiment was set out following a randomized complete block design with eight treatments (*T. chilonis* eggs released at three rates, four different insecticides and a control) replicated three times. The whole trial therefore consisted of 24 experimental plots; each plot was 5 x 3 m and consisted of about 110 plants. Non-experimental plots were placed surrounding the entire trial to remove edge effects.

Release of Trichogramma chilonis

Cards each containing 1500 eggs of *Trichogramma chilonis* were acquired from the Plant Protection Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The cards were cut down into four equal pieces and glued onto plants at five randomly different positions in the plot at three different application rates: 60,000, 80,000 and 1,00,000 eggs acre^{-1}. The *T. chilonis* egg releases were done twice. Initially, 51 days after sowing followed by another one 72 days after sowing. Each plot was covered with a nylon net barrier (2.4 m tall, 150 mesh) to help prevent the parasitoids from moving between plots. The data on percent parasitism were recorded five days after each release of the parasitoid eggs. The eggs which went black in colour were considered as parasitized, while the rest (hatched or otherwise desiccated) were considered as non-parasitized.

Insecticides

The following commercial formulations of new insecticides used in the experiment were: Lannate, Proclaim, Steward and Tracer. Active ingredients and formulation details for each product are given in Table 1.

Larval population and yield assessment

Two sprays of the insecticides were applied after the first emergence of *H. armigera* on the sunflower crop. The first application of treatments was done 51 days after sowing followed by the second one 72 days after sowing (DAS). The larval population of *H. armigera* was assessed by counting the total number of larvae per plant on five randomly selected plants in each plot, five days after each spray. Per plot seed yield for all the treatments was recorded at maturity and converted to yield in kg acre^{-1}.

Data analysis

Egg parasitism, larval population and yield differences between treatments were determined through two-way ANOVAs following a randomized complete block design using MINITAB

statistical software (Steel *et al*., 1990). Means were separated by using the Tukey-Kramer (HSD) test, at *P = 0.05* (Sokal and Rohlf, 1995).

Table 1. Insecticide products, active ingredients and formulations used in the experiments

Trade name	Active ingredient	Formulation
Lannate	methomyl	400 g kg^{-1} soluble powder
Proclaim	emmamectin benzoate	19.2 g l^{-1} emulsifiable concentrate
Tracer	spinosad	240 g l^{-1} suspension concentrate
Steward	indoxacarb	150 g l^{-1} suspension concentrate

Results and discussion

***Egg parasitism of* Helicoverpa armigera *by* Trichogramma chilonis**

The parasitization of *H. armigera* eggs by *T. chilonis* differed significantly among treatments ($F_{2,8}$ = 15843.86; $P < 0.005$) (Figure 1). Percentage egg parasitism increased with the number of eggs released per acre. The maximum parasitism rate was 81.42%, when 1,00,000 eggs of the parasitoid were released $acre^{-1}$ followed by 69.13% (at 80,000 eggs $acre^{-1}$) and 53.28% (at 60,000 eggs per $acre^{-1}$).

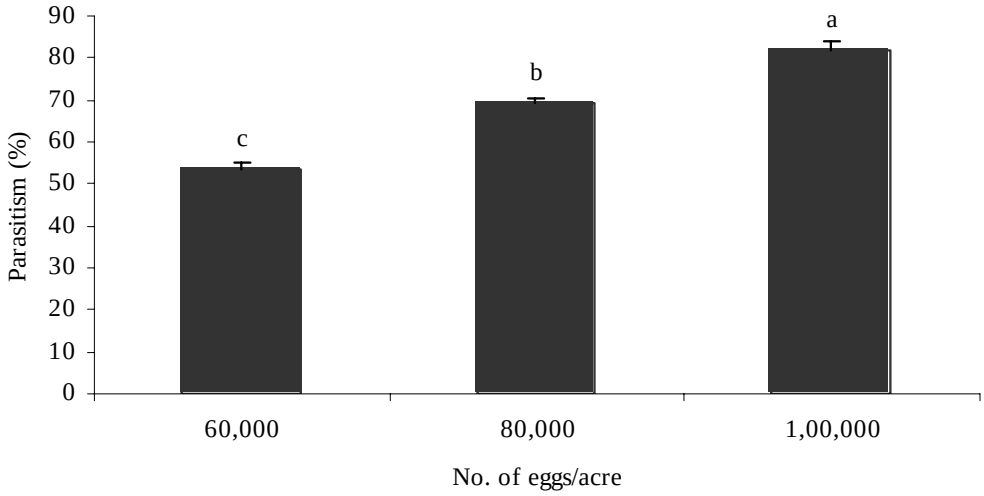


Figure 1. Egg parasitism (%) of *Helicoverpa armigera* by *Trichogramma chilonis*

Field experiments on different crops have demonstrated that *T. chilonis* can be effective against *H. armigera.* For example, in Mexico, release of *Trichogramma* spp. on chickpea led to a reduction of both *Heliothis* and *Spodoptera* spp. (Castanos, 1979) and in sorghum more than 26% eggs of *H. armigera* were parasitized (Bhatnagar *et al.*, 1982). Tests conducted in cotton indicated a reduction in *H. armigera* larval population as a consequence of parasitization by *Trichogramma* parasitoids (King *et al.*, 1982). Similarly, a high level of egg

parasitism (63%) has been achieved in *H. armigera* by *T. chilonis* and *T. achaea* in cotton under field conditions (Reddy and Manjunatha, 1999) and 75.6% parasitization was observed in the laboratory conditions (Reddy and Manjunatha, 2000). The results of the present experiment indicate that the percentage of *H. armigera* egg parasitism in sunflower increased corresponding to the increase in the number of eggs released acre^{-1}. However, *T. chilonis* alone may not be potent enough to control sunflower head-worm adequately. It has been reported that when *T. chilonis* eggs were released at 30 cards ha^{-1} *H. armigera* on chickpea was not adequately controlled (Wakil, 2004). Similarly, Romeis *et al.* (1999) and Tiwari *et al.* (2002) reported that *T. chilonis* failed to parasitize *H. armigera*.

***Effect of insecticides and* Trichogramma chilonis *on the larval population of* Helicoverpa armigera**

The larval population differed significantly among the treatments ($F_{7,23}$ = 52.36; P < 0.005) (Figure 2). When the new insecticides and *T. chilonis* were tested, the larval population of *H. armigera*, ranged from 0.57 to 0.28 per plant compared with 0.97 larvae per plant in the control. The lowest larval population was rerecorded in Steward (0.28) followed by 1,00,000 eggs acre^{-1} of *T. chilonis* (0.29), Tracer (0.32), Procliam (0.34), Lannate (0.41), 80,000 eggs acre^{-1} of *T. chilonis* (0.49) and 60,000 eggs acre^{-1} of *T. chilonis* (0.57).

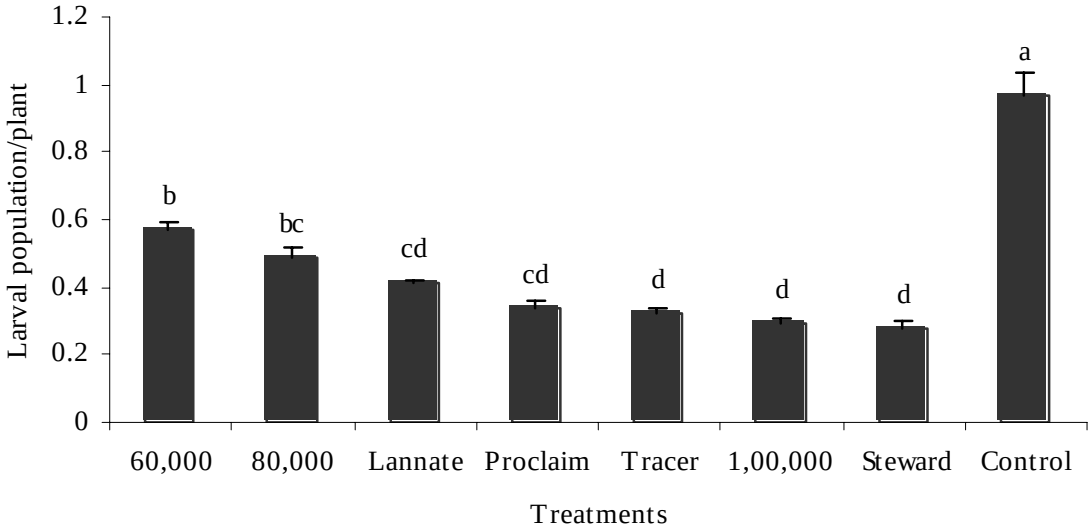


Figure 2. Larval population of *Helicoverpa armigera* plant^{-1} as effected by different treatments

When chemical insecticides such as Lannate, Proclaim, Tracer and Steward were used at recommended dosages, there was a highly significant reduction in the larval population compared with the control. These results and our aim of applying chemical pesticides was agreeable with Kohli (1982) who reported that synthetic pyrethroids can be used as part of an integrated control program in which their low toxicity to predators and their irritant effects on mobile parasitoids and pollinators are of major importance. Indoxacarb and spinosad performed well and demonstrated their robustness even at rates of 50% or less of the registered cotton rates for *Helicoverpa* spp. (Schulze and Tomkins, 2002) and in another study indoxacarb resulted in 70% mortality of *H. armigera* under laboratory conditions at the lowest dose of 10 g a.i. ha^{-1} (Ramasubramanian and Requpathy, 2004). Our results also showed that the indoxacarb (Steward) and spinosad (Tracer) even at the lowest rates gave the minimum

larval population as compared with the control plots and also gave the highest yields of sunflower. At the efficacious rate tested on sunflower, both indoxacarb and spinosad would be affordable products. Furthermore, indoxacarb and spinosad are generally more selective and result in less disruption of natural enemies than pyrethroids and carbamates (Wilson *et al*., 1999). These products would fit well with grain IPM programs that aim to enhance the activity of natural enemies.

The other products tested in the present studies (emmamectin benzoate (Proclaim) and methomyl (Lannate)) show promise both in reducing the pest larval population and improving yield in sunflower, but further testing is warranted to establish their efficacy, particularly in terms of time to kill and crop damage. The delay in reduction of larval densities following treatment, particularly with methomyl (Lannate) in sunflower, is the most serious barrier to its acceptance by pest managers.

Effect of insecticides and* Trichogramma chilonis *on yield

Overall, the yield differed significantly between treatments ($F_{7,23}$ = 4492.39; P < 0.005) (Figure 3). The yield obtained in the different treatments ranged between 243 to 643 kg acre^{-1}. The maximum yield (643 kg acre^{-1}) was observed with the application of Steward and was not statistically different from the yield obtained when 1,00,000 *T. chilonis* eggs were released. The minimum yield (352 kg acre^{-1}) was obtained when 60,000 eggs were released, but even this was significantly greater than the control.

In conclusion, our data indicates that the four insecticides tested and the parasitoid *T. chilonis* can provide protection against the sunflower head-worm. In general, pesticide applications decreased populations of the head-worm more effectively compared to the parasitoid alone, if applied at lower rates, and can gave higher yield. On the other hand, the parasitoids may effectively manage the head-worm populations when applied at very high rates (100,000 eggs acre^{-1}). However their use may become more effective in lower amounts when used along with the new synthetic insecticides. Use of natural biocontrol agents such as *T. chilonis* may help to reduce the amount of insecticides used in sunflower production, and lower the risk of resistance and the hazards arising from the continuous use of conventional insecticides.

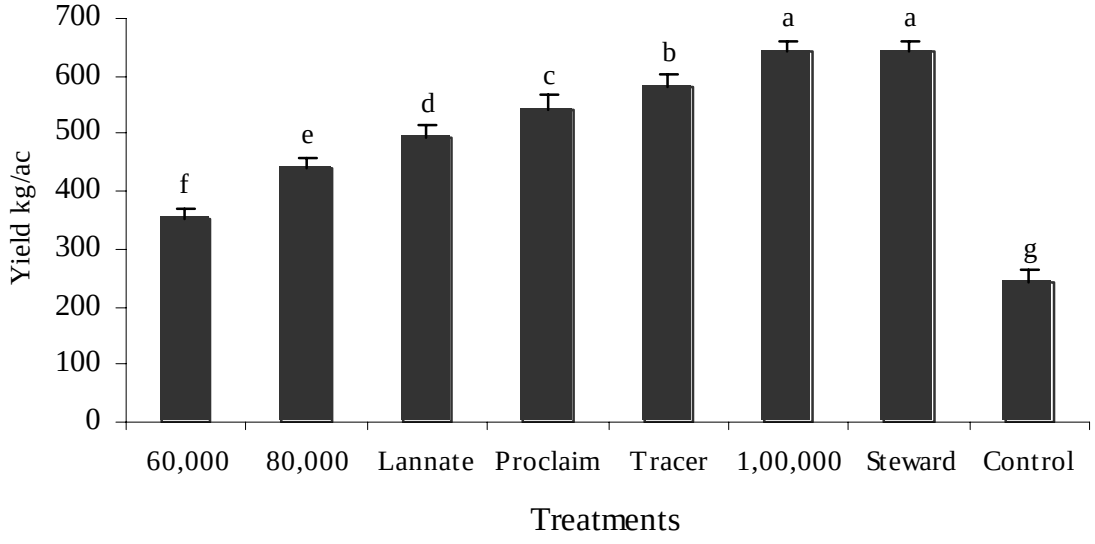


Figure 3. Yield (kg acre^{-1}) of sunflower in different treatments

Acknowledgements

The authors are grateful to the editors: Drs. Sam Cook, Bernd Ulber & Birger Koopmann. Special thanks are extended to the University of Arid Agriculture, Rawalpindi, Pakistan for providing an experiment site on the research farm.

References

Ahmad, M., Arif, M.I. & Ahmad, Z. 1995: Monitoring insecticide resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. – J. Econ. Entomol. 88: 771-776.

Ahmad, M., Arif, M.I. & Attique, M.R. 1997: Pyrethroid resistance of *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. – Bull. Entomol. Res. 87: 343-347.

Ahmad, M., Arif, M.I., Ahmad, Z. & Attique, M.R. 1998a: *Helicoverpa armigera* resistance to insecticides in Pakistan. – In: Proceedings of Belwide Cotton Conferences 1138-1140.

Ahmad, M., Arif, M.I. & Ahmad, Z. 1998b: Analysis of pyrethroid resistance in *Helicoverpa armigera* in Pakistan. – In: Proceedings of the Second World Cotton Research Conferences, Athens, Greece.

Ahmad, M., Arif, M.I. & Ahmad, Z. 1999: Patterns of resistance to organophosphate insecticides in field populations of *Helicoverpa armigera* in Pakistan. – Pestic. Sci. 55: 626-632.

Ahmad, M., Arif, M.I. & Ahmad, Z. 2001: Resistance to carbamate insecticides in *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Pakistan. – Crop Prot. 20: 427-432.

Amjad, M, Haq, I. & Ali, N. 2005: A key to insect pest management of sunflower crops. – Oilseed Research Programme, National Agricultural Research Centre, Islamabad, Pakistan.

Bhatnagar, V.S., Lateef, S.S., Sithanantham, S., Pawar, C.S. & Reed, W. 1982: Research on *Heliothis* at ICRISAT. – *In* Proceedings of the International workshop on *Heliothis* Management, 15-20 November, 1981. ICRISAT Center, Patancheru, AP, India. 385-396.

Castanos Lugo, C. 1979: Control integrado de plagas en el cultivo de garbanzo. – In: Reunion nacional de control biologico, pp. 164-167. Veracruz, Mexico, Comite Organizador de la VII Reunion Nacional de Control Biologico.

Fitt, G.P. 1989: The ecology of *Heliothis* in relation to agro ecosystems. – Ann. Rev. Entomol. 7: 685-688.

Greathead, D.J. & Girling, D.J. 1982: Possibility for natural enemies in *Heliothis* management and the contribution of the Commonwealth Institute of Biological Control. – In: Proceedings of the International Workshop in *Heliothis* Management, 15-20 November, 1981. Patancheru, India. ICRISAT, eds. Reed, W. & Kumble, V.: 147-158.

King, E.G., & Coleman, R.J. 1989: Potential for biological control of *Heliothis* species. – Ann. Rev. Entomol. 34: 53-75.

King, E.G., Powell, J.E. & Smith, J.W. 1982: Prospects for utilization of parasites and predators for management of *Heliothis* spp. – In: Proceedings of the International Workshop in *Heliothis* Management, 15-20 November, 1981. Patancheru, India. ICRISAT, eds. Reed, W. & Kumble, V.: 103-122.

Kohli, A. 1982: The likely impact of synthetic pyrethroids of *Heliothis* management. – In: Proceedings of the International Workshop in *Heliothis* Management, 15-20 November, 1981. Patancheru, India. ICRISAT, eds. Reed, W. & Kumble, V.: 197-205.

Nagarkatti, S. 1982: The utilization of biological control in *Heliothis* management in India. – In: Proceedings of the International Workshop in *Heliothis* Management, 15-20 November, 1981. Patancheru, India. ICRISAT, eds. Reed, W. & Kumble, V.: 159-167.

Ramasubramanian, T. & Requpathy, A. 2004: Evaluation of indoxacarb against pyrethroid resistance population of *Helicoverpa armigera* Hub. – J. Entomol. 1(1): 21-23.

Reddy, G.V.P. & Manjunatha, M. 1999: Influence of the host plants on parasitism of *Helicoverpa armigera* (Lepidoptera: Noctuidae) by two egg parasitoids, *Trichogramma chilonis* and *Trichogramma achaea* (Hymenoptera: Trichogrammatidae). – Internat. Pest. Contr. 41: 223-225.

Reddy, G.V.P. & Manjunatha, M. 2000: Laboratory and field studies on the integrated pest management of *Helicoverpa armigera* (Hübner) in cotton, based on pheromone trap catch threshold level. – J. Appl. Entomol. 124: 213-221.

Romeis, J., Shanower, T.G. & Zebitz, C.P.W. 1999: Why *Trichogramma* (Hymenoptera: Trichogrammatidae) egg parasitoids of *Helicoverpa armigera* (Lepidoptera: Noctuidae) fail in chickpea. – Bull. Entomol. Res. 89(1): 89-95.

Schulze, K.J. & Tomkins, A.R. 2002: Cotton Pest Management Guide 2002-2003. – NSW Agriculture/Australian Cotton Corporative Research Centre, Narrabri, Australia.

Sokal, R.R. & Rohlf, F.J. 1995: Biometry. 3rd edition. – Freedman and Company, New York.

Steel, R.G.D., Torrie, J.H. & Dickey, D.A. 1990: Principles and Procedures of Statistics: A Biometrical Approach. 3rd edition. – WCB McGraw Hill Companies, Inc., USA.

Tiwari, S., Khan, M.A., Meena, A. & Agnihotri, M. 2002: Relative efficacy of *Trichogramma chilonis* Ishii on two varieties of chickpea under Pantnagar conditions to control *Helicoverpa armigera* Hübner. – Shashpa. 9(1): 57-60.

Wakil, W. 2004. Management of *Helicoverpa armigera* (Hüb.) in chickpea (*Cicer arietinum* L.) under rainfed areas of Punjab, Pakistan. – Ph.D. Thesis. Department of Agricultural Entomology, Faculty of Agriculture, University of Agriculture, Faisalabad, Pakistan.

Wilson, L., Holloway, J., Mensah, R. & Murray, D. 1999: Impact of insecticides and miticides on predators in cotton. Support Document 1. – In: Mensah, R. & Wilson, L. (eds.). Integrated Pest Management Guidelines for Australian Cotton. Australian Cotton Cooperative Research Centre, Narrabri, Australia,

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 125-130

Subsequent effect of tau-fluvalinate (Maverik) and lambda-cyhalotrine (Karate) pyrethroids on the activity of carabid beetles (Coleoptera: Carabidae) in winter oilseed rape

Zdzisław Klukowski, Jacek P. Twardowski and Małgorzata Irzykowicz
Agricultural University, Department of Plant Protection, Cybulskiego 32, 50-205 Wrocław, Poland

Abstract: Carabid beetles are important in agrocenosis as predaceous insects of some pests of winter oilseed rape, such as *Meligethes aeneus*, *Ceuthorhynchus pallidactylus*, *C. napi*, and *Dasyneura brassicae*. This study determined the effect of non-selective pyrethroids on reducing the number of phytophagous larvae (as a food) on the total amount and species diversity of carabid beetles. The experiment was carried out in 2002 and 2004 at Experimental Station Pawłowice (Agricultural University at Wrocław). Each year, the insecticides lambda-cyhalotrine (Karate 25 EC) and tau-fluvalinate (Mavrik 2S) were applied to control major pests larvae of the rapeseed crop. Data from two years of investigations prove indirectly the effect of pyrethroids application on dynamic seasonal appearance of beetles in winter rapeseed crop.

Key words: Pyrethroids, Carabidae, carabid beetles, winter rapeseed, subsequent effect

Introduction

Carabid beetles (Coleoptera: Carabidae) are an important group of arthropod predators in agroecosystems and have great potential to reduce pests (Thiele, 1977; Sunderland, 2002). The effect of different agricultural practices such as soil cultivation, crop types, fertilization and pesticide application on this epigeal group of arthropods is well studied (Holland & Luff, 2000; Hance, 2002). However, the understanding on the subsequent effect of insecticides on carabids seems to be unsatisfactory. The majority of species are generalists, feeding on different groups of organisms (Hengeveld, 1980), including oilseed rape pests such as pollen beetles (*Meligethes aeneus* F.), cabbage stem weevils (*Ceutorhynchus pallidactylus* Marsh., *C. napi* Gyll.) and brassica pod midge (*Dasyneura brassicae* Winn) (Büchs & Nuss, 2000). Larvae of these pests are attractive and easily accessible food, especially when they leave the plant stems or flowers and drop to the soil to pupae. Therefore, application of insecticides at these times of larval activity can cause subsequent effects for carabids.

The main objective of this study was to determine how non-selective insecticides which control the number of oilseed rape pests can influence the species composition and/or abundance of carabid beetles.

Materials and methods

The study was carried out at the Agricultural Experimental Station in Pawłowice near Wrocław, Lower Silesia, Poland in 2002 and 2004. The experiment was set up using 12 plots of oilseed rape (30 x 30 m) in a random design with four replicates to insecticide treatments and associated controls. Insecticides (lambda-cyhalotrine – Karate 25 EC and tau-fluvalinate – Mavrik 2S) at the full dose rate, were applied simultaneously at growth stage 63-68 (BBCH) on relevant plots of each replicate.

To determine the number of larvae of the oilseed rape pests which dropped to the soil to pupate, water traps (0.2 m^2) placed on the ground were used. Pitfall traps were used to sample carabid activity. Two water traps and one pitfall trap were placed in each plot. Capture results were analyzed over a three-week duration before pyrethroids application, with the aim of design standardization. The number of beetles noted from the plots during this period was similar, thus excluding the possibility of strong influences of factors other than treatment effects on the experimental design (i.e. spatial distribution of beetles). Pitfall and water traps were placed again on the plots on the second day after insecticide treatment and beetles were collected over the following 6 weeks. The numbers of predatory beetles collected from each plot during the sampling time were analyzed by principal component analysis (PCA) separately for each treatment.

Results and discussion

The analysis of dynamic population parameters for the most numerous carabids shows strong evidence of an effect on the behavior of the predators in response to the knock-down effects of the insecticides on pest larvae. The large amount of food available in the shorter than usual time stimulated predatory species to shift the dynamics of their own appearance earlier or later in comparison to untreated plot (Table 1). The disruptive effect of pyrethroids displays a low correlation coefficient with unsprayed plot (Table 1).

Table 1. The dynamic population parameters of the most numerous predatory carabid beetles after knock-down effects on their prey following treatment with tau-fluvalinate (Maverik) and lambda-cyhalotrine (Karate) pyrethroids

Species	Total no. of beetles caught in 6 weeks after treatment	Date of maximum number of species			Mean no. of beetles (per trap) caught over untreated plot in 2-8 days after pyrethroid application		Correlation of untreated plot and plot treated by:	
		Untreated	Karate	Mavrik	Karate	Mavrik	Karate	Mavrik
2002								
Poecilus cupreus	330	24.06	17.06	17.06	3.00	-0.75	0.53*	0.33
Pterostichus melanarius	414	17.06	24.06	17.06	0	0.25	0.48*	0.65*
Ophonus brevicollis	620	27.05	24.06	27.05	-3.75	-6.50	-0.37	0.12
2004								
Poecilus cupreus	1401	3.06	7.06	3.06	-1.75	6	0.75	0.91*
Pterostichus melanarius	441	14.06	28.06	14.06	0	0	0.87*	0.90*
Ophonus brevicollis	108	21.06	3.06	23.05	1	2.5	-0.50	-0.30

* asterisk indicates statistical significance ($p<0.05$)

Relationships between carabid species have been described by Principal Component Analysis (PCA) in both years in all treatments (Figure 1a-f).

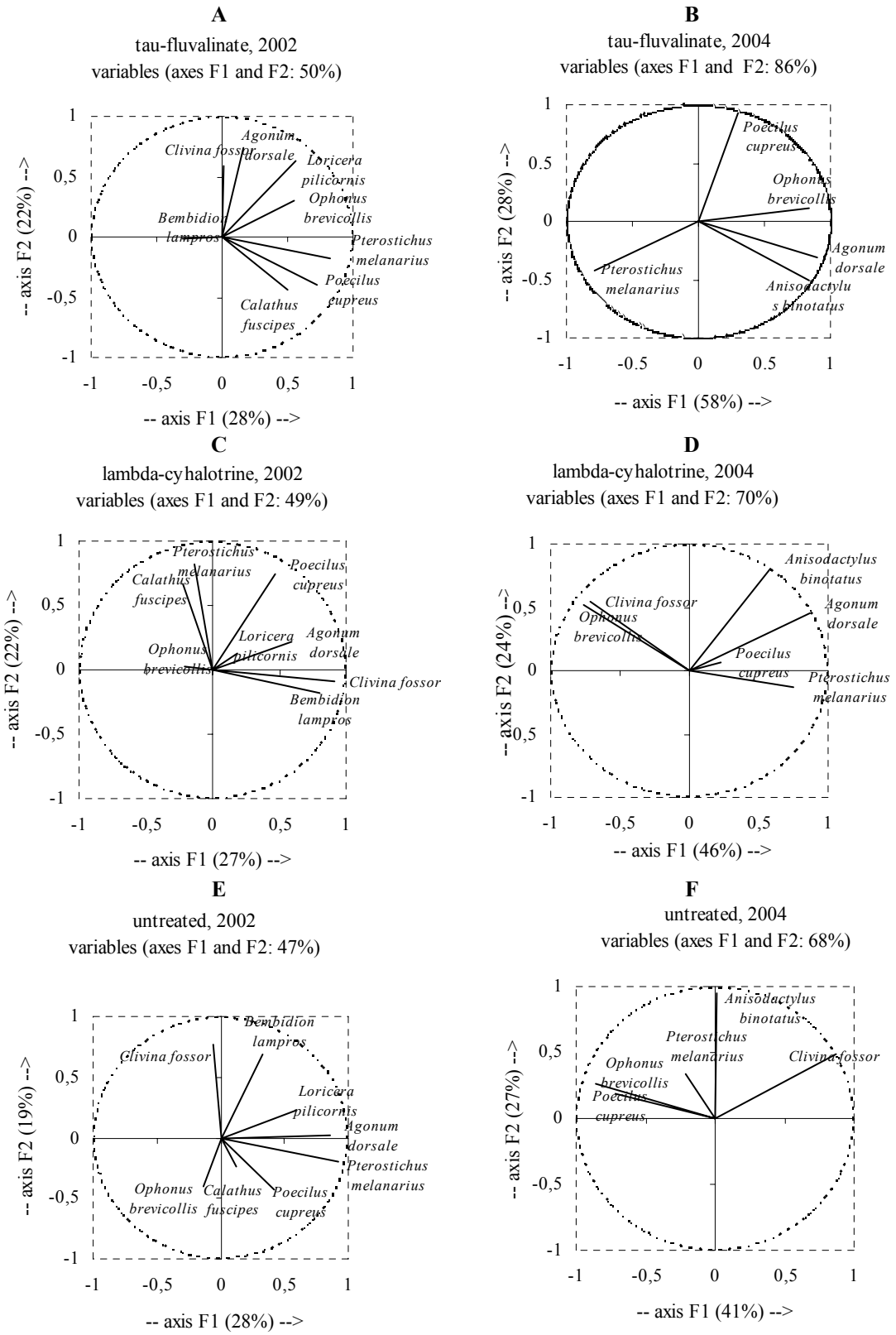


Figure 1. Correlation of the most numerous species of predatory ground beetles on fields treated by tau-fluvalinate (Maverik) and lambda-cyhalotrine (Karate) pyrethroids and untreated fields in 2002 and 2004 (Principal Component Analysis, PCA)

The predatory species comprised 44% of all carabids. The undisturbed patterns of appearance of these beetles are derived from the untreated plots (Fig. 1e-f), which can be compared to both types of insecticide-treated plots (Fig. 1a-d). The narrower the angle between two species' line inside of each circle, the more similar to each other they are in terms of the dynamics of their activity in response to that treatment.

Results from the investigations conducted in two years indirectly prove the effect of pyrethroids on seasonal appearance of carabid beetles in winter rapeseed crop agrocenosis. Increased mortality of phytophagous larvae caused through application of insecticides was found (Fig. 2 a & b).

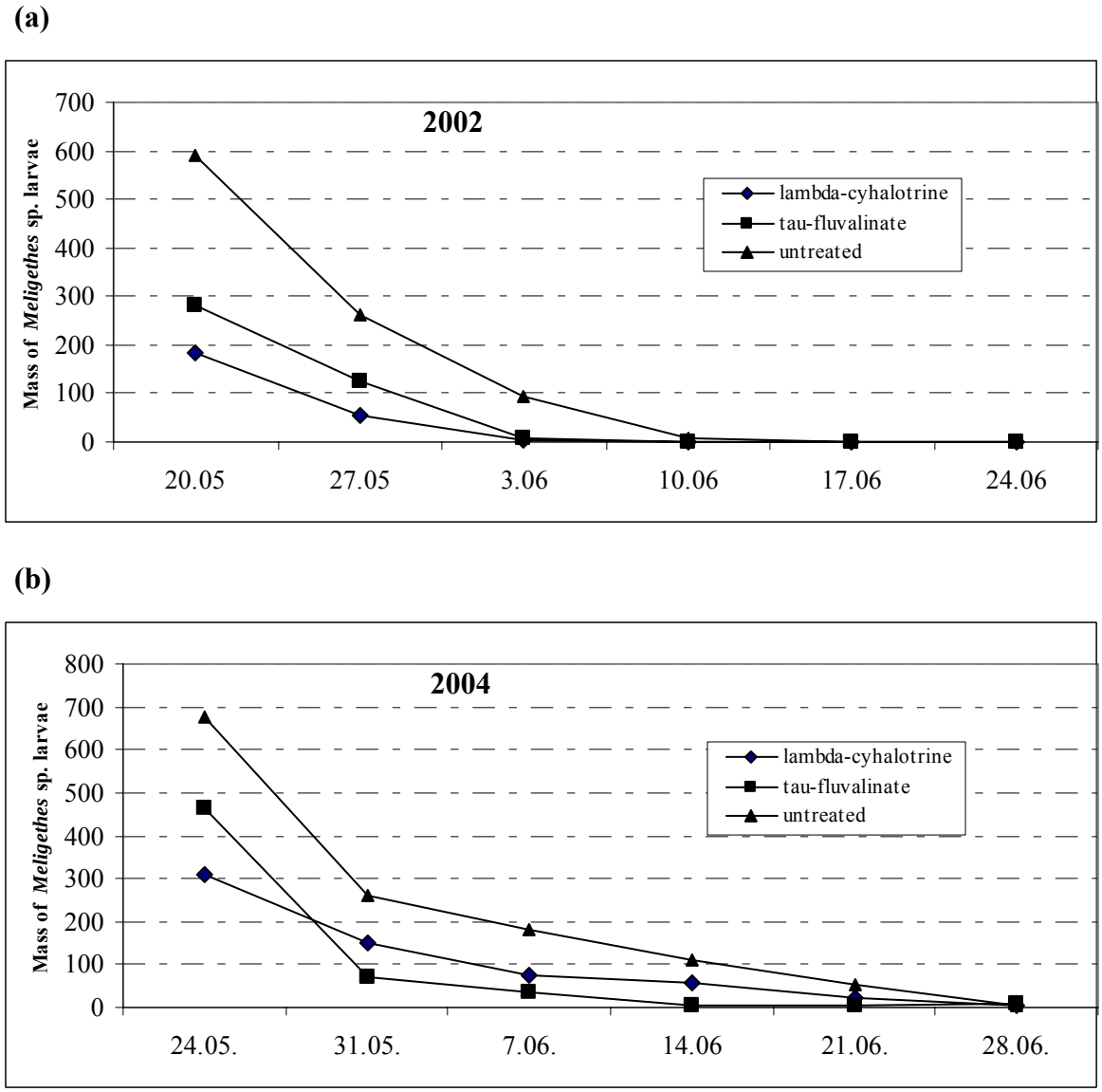


Figure 2. Mean larval mass (mg/m^2) of *Meligethes* sp. falling from oilseed rape plants with different treatments that were caught in water traps placed on the ground in 2002 (a) and 2004 (b).

In the first week capture (20.05.2002 and 24.05.2004), 31% and 45% more larvae were caught after lambda-cyhalotrine application, respectively and 48% and 68% after tau-fluvalinate application compared to the untreated plots, respectively. This suggests a more rapid knock-down effect by lambda-cyhalotrine than tau-fluvalinate. This can also be seen by comparing the slope of the graphs between the first two dates. This effect for both insecticide treatments decreases the total available mass of larvae as food for Carabids. Also, the time of the larval food availability for predators was longest on untreated plots. The knock-down effect of the insecticides therefore reduces the window of food availability. This also affected the intraspecific relationship of predaceous Carabidae.

Many researchers are pointing out the effect of impoverishing the carabid fauna on fields treated by insecticides (Berraondo *et al*., 1998; Lee *et al*., 2001; Kaczmarek, 1992). However, quantitative analysis of carabids does not always show these effets. Maximal difference in numbers of beetles between treatments in our experiment was found in the first week after spray, but they were still statistically not significant from the untreated plots (Table 1). The lack of sharp quantitative responses of carabids after pyrethroid applications has been noted previously (Nilsson & Andreasson, 1987; Drzewiecki & Sokołowski, 1997).

A higher number of beetles caught in the first week after the pyrethroids application was sometimes observed in treated than untreated plots (e.g. 6 examples/plot of *Poecilus cupreus* over number in the untreated plots in 2004). This was probably caused by their increased activity (Chiverton, 1984). This activity can be linked with non-lethal contact with insecticides. Later on after application a reduction in the prey population, resulting in the deficiency of sustenance, would also increase activity though increased searching behaviour in foraging.

In this study we demonstrate that the interdependency/relationship of functioning of the most numerous predatory carabid species in an undisturbed "nutritional niche" (non treated plots) clearly diverges from that on treated plots (Fig 1a-d; 1e-f). This can also be seen as a specific changed relationship between the most numerous predatory species penetrating treated plots and particular biologically active compounds. From that point of view, tau-fluvalinate (Maverick) seems to be slightly less disruptive (i.e. has a better correlation coefficient with untreated controls) than lambda-cyhalotrine (Karate) (Table 1).

Acknowledgements

This work was conducted as part of the ‘Integrated pest management strategies incorporating bio-control for European oilseed rape pests’ project supported by the EU (MASTER-QLK5-CT-2001-1447).

References

Berraondo, P.E.Z., Morgan, D., Walters, K.F.A., Young, J.E.B. & Lane, A. 1998: Spatial recovery of two species of *Carabidae* following cumulative pesticide applications in winter wheat. – BCPC - Pests & Diseases: 613-618.

Büchs, W. & Nuss, H. 2000: First steps to assess the importance of epigeic active polyphagous predators on oilseed rape insect pests with soil pupating larvae. – IOBC/ wprs Bulletin 23(6): 151-163.

Chiverton, P.A. 1984: Pitfall-trap catches of the carabid beetle *Pterostichus melanarius*, in relation to gut contents and prey densities in insecticide treated and untreated spring barley. – Entomogia Experimentalis et Applicata 36: 23-30.

Drzewiecki, S. & Sokołowski, A. 1997: Fauna stawonogów naziemnych w uprawach pszenicy ozimej objętych integrowanym programem ochrony roślin. – Progress in Plant Protection 37: 127-130.

Hance, T. 2002: Impact of cultivation and crop husbandry practices. – In: J.M. Holland (ed.): The Agroecology of Carabid Beetles: 231-249.

Hengeveld, R. 1980: Polyphagy, oligophagy and food specialization in ground beetles (Coleoptera, Carabidae). – Netherland Journal of Zoology 30: 564-584.

Holland, J.M. & Luff, M.L. 2000: The effects of agricultural practices on Carabidae in temperate agroecosystems. – Integrated Pest Management Reviews 5: 109-129.

Karczmarek, S. 1992: Wpływ preparatu Decis 2,5 EC na Carabidae w uprawie ziemniaka. – Polskie Pismo Entomologiczne 61: 125-129.

Lee, J.C., Menalled, F.D. & Landis, D.A. 2001: Refuge habitats modify impact of insecticide disturbance on carabid beetle communities. – Journal of Applied Ecology 38: 472-483.

Nilsson, C. & Andreasson, B. 1987: Parasitoids and predators attacking pollen beetles (*Meligethes aeneus* F.) in spring and winter rape in southern Sweden. – IOBC/wprs Bulletin 10(4): 64-73.

Sunderland, K. 2002: Invertebrate pest control by carabids. – In: J.M. Holland (ed.): The Agroecology of Carabid Beetles: 165-214.

Thiele, H.U. 1977: Carabid beetles in their environments. – Berlin, Heidelberg, New York. Springer-Verlag.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 131-134

First steps to analyse pyrethroid resistance of different oilseed rape pests in Germany: An extended abstract

Udo Heimbach[1], Andreas Müller[1] and Thomas Thieme[2]
[1] *BBA, Institute for Plant Protection in Arable Crops and Grassland, Messeweg 11/12, D-38104 Braunschweig*
[2] *BTL Bio-Test Labor GmbH, Birkenallee 19, D-18184 Sagerheide*

Abstract: Due to EU regulations of plant protection products and increasing demands for human and environmental safety issues the number of active substances which can be used to control pest insects was reduced in the last year. In Germany only pyrethroids are available for the control of most pest insects in oilseed rape. Therefore resistance development of pest insects to pyrethroids is becoming more relevant because the resistance status of pest insects need to be known for IPM.

In the past years Meligethes aeneus has developed resistance to pesticides in different European regions. Actually resistant *M. aeneus* populations seem to spread in Europe. No information on possible development of resistance to the other pest insects of rape is available, though they often are exposed to more than one pyrethroid application per season similar to *M. aeneus*.

We conducted laboratory tests using active substances of pyrethroids on different pest insects of rape collected in different areas of Germany. Tests have been carried out on *Phyllotreta* spp., *Ceutorhynchus assimilis, C. quadridens, C. napi* until now.

Results obtained and methods used will be presented and discussed.

Key words: Pyrethroid resistance, oilseed rape pests, *Ceutorhynchus assimilis, C. quadridens, C. napi*

Introduction

Due to EU regulations of plant protection products and increasing demands for human and environmental safety issues, the number of active substances which can be used to control pest insects were reduced in the past few years in the EU. In Germany at the moment, only pyrethroids are available for the control of most pest insects in oilseed rape. Therefore resistance development of pest insects to pyrethroids is very relevant for IPM. In the past few years, *Meligethes aeneus* has developed resistance to pyrethroids in different European regions and resistant *M. aeneus* populations seem to be spreading also in Germany. No information on possible development of resistance to the other pest insects of rape is available, though they often are exposed to more than one pyrethroid application per season, similar to *M. aeneus*. According to EU pesticide regulation, resistance issues need to be addressed during the registration process of pesticides. To foster sensitivity testing and method development as well as to get more knowledge on the resistance status of oilseed rape pest insects, a resistance monitoring program for most relevant pest insects in oilseed rape was started in Germany.

Materials and methods

Sampling of the different oilseed rape pests

Species collected were *Ceutorhynchus assimilis*, *C. napi*, *C. pallidactylus*, *Dasineura brassicae*, *Meligethes aeneus*, and *Phyllotreta* spp. All species except *D. brassicae* were collected in oilseed rape fields in Germany by either direct hand collection, sweep netting or yellow

water trapping. For the collection of *D. brassicae*, infested pods of oilseed rape were collected and kept in hatching cages in the laboratory. Larvae leaving the pods pupated in a small amount of soil supplied below the pods. Hatched midges were kept only for up to 3 days at low temperature and high humidity before they were used in the tests. All insects which were not collected by the BBA were mailed to Braunschweig for the tests. Most samples arrived in good condition; only 4 out of 38 mailings could not be used for tests due to poor condition.

Insecticide tests in the laboratory, adult-vial-test

Glass vials (30 ml) were used for the test. Prior to testing, the vials were coated with the active substance of pyrethroids (lambda-cyhalothrin and cypermethrin dissolved in acetone). As far as possible, besides a control, several rates of the pyrethroids were tested, depending on the number of insects available. Rates used for λ-cyhalothrin were: 0.075 µg/cm² of glass surface (representing the registered field rate in Germany of 7.5 g a.s./ha) and lower (down to 0.00075 µg/cm²) and higher rates (up to 0.75 µg/cm²). The rate of 0.015 µg/cm² was chosen to distinguish differences in population sensitivity, because at this rate all samples of *M. aeneus* with known field resistance showed less than 100% mortality 5 hours after exposure. If possible, up to 5 replications were carried out per test rate. Assessments were carried out after 1, 5, and 24 hours. Assessments after 5 hours were chosen to be reported because control mortality was often increased at 24 hours, and a significant difference between treatments was detectable between 1 and 5 hours; only a small further increase in mortality between 5 and 24 hours was seen, which is the expectation for the fast acting pyrethroids. All results presented were not corrected for control mortality values.

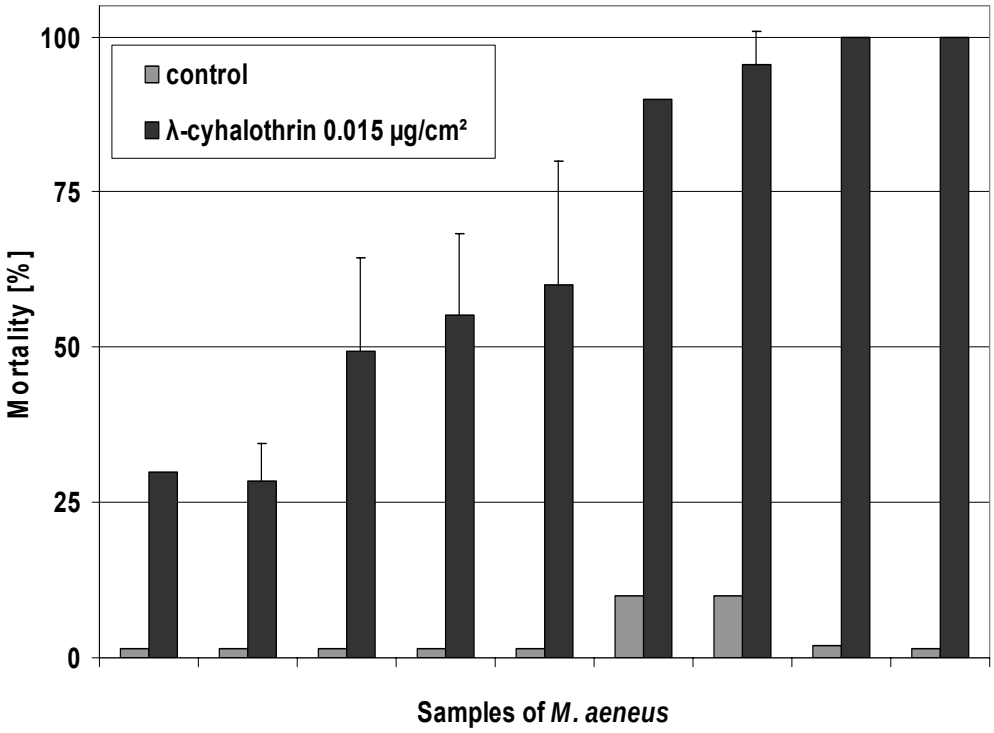


Figure 1. Mortality (mean ± SD) of several *M. aeneus* samples in adult-vial-tests after 5 hours exposure to λ-cyhalothrin at a rate of 0.015 µg a.s./cm².

Results and discussion

The effects of λ-cyhalothrin at a rate of 0.015 µg/cm² clearly show differences between the nine samples of *M. aeneus* tested. The samples originating from regions or fields with known pyrethroid resistance react differently to those from areas where no resistance is yet known. These differences at a rate of 0.015 µg/cm² seem to allow separation between populations that are still sensitive and those with detectable reduction of efficacy in the field (Fig. 1). Both pyrethroids tested showed reduced effects when resistant populations were tested.

Especially from northern Germany, the region with most pyrethroid resistance problems, only few pest insects samples except *M. aeneus* could be tested, because of low pest density in 2005 in this region. Results of two samples of stem weevils (No. 13 and 16) indicate that there might be a rising problem with this species in some areas. But to be sure if there is resistance developing or not, laboratory data generally need to be validated with real field data.

More detailed results and information on the monitoring will be published in: Heimbach et al. 2006.

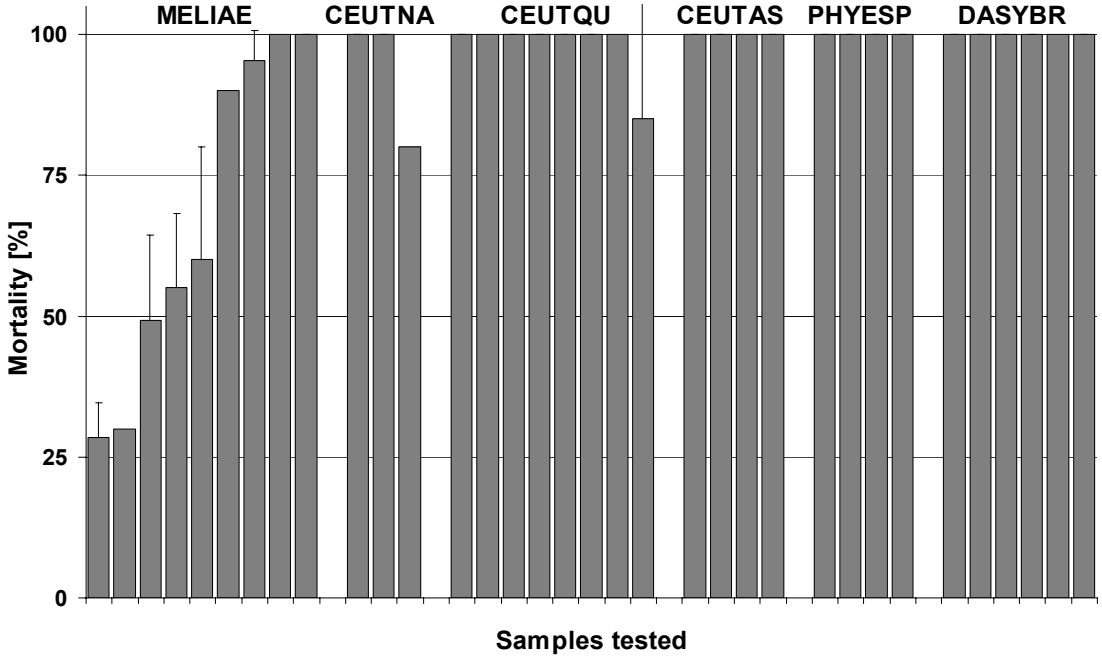


Figure 2. Mortality (mean ± SD) of insect samples after 5 hours exposure to 0.015 µg/cm² λ-cyhalothrin. Species tested: *M. aeneus* (MELIAE), *C. napi* (CEUTNA), *C. pallidactylus* (CEUTQU), *C. assimilis* (CEUTAS), *Phyllotreta* spp. (PHYLSP), *D. brassicae* (DASYBR).

Conclusions

Field tests are too time consuming and expensive to screen for resistance. Therefore adequate and standardised laboratory tests should be developed which are validated by field experiences. These can then be easily used in cases of field failure of a product to decide if resistance or something else caused the problem. Sensitivity data of laboratory tests can support the decision if there is resistance in a population or not. Distinct differences in the results of laboratory tests between different populations from different regions indicate possible resistance. The method

presented here seems to be able to analyse increasing and already existing resistance problems with pyrethroids in oilseed rape. It also needs to be checked carefully, if other pest insects (e.g. aphids - probably not) can be tested in this way.

Acknowledgements

We thank all collectors of samples (Bergmann, Burghause, Eiselt, Goebel, Hartmann, Hemmer, Kaak, Landschreiber, Landvogt, Rehm, Rüdinger, Rupprecht, Schackmann, Schröder, Seifert, Thalhammer, Ulber, Wedemeyer) for supporting the monitoring, the UFOP for a financial contribution, and the companies BASF and Syngenta for supply of the active substances.

References

Heimbach, U., Müller, A., & Thieme T. 2006: First steps to analyse pyrethroid resistance of different oilseed rape pests in Germany. – Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 58: 1-5.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 135-140

Resistance of pollen beetle *(Meligethes aeneus* F.) to pyrethroids, chloronicotinyls and organophosphorous insecticides in Poland

Paweł Węgorek and Joanna Zamojska
Institute of Plant Protection, Miczurina 20, 60-318 Poznań, Poland

Abstract: Poland is a major producer of oilseed rape (*Brassica napus*). The pollen beetle (PB) (*Meligethes aeneus*) is the most serious oilseed rape pest in Poland and is considered to be the pest with the highest likelihood of developing insecticide resistance (Ląkocy, 1967; Węgorek, 2005). Pyrethroid, organophosphorous and chloronicotinyl insecticides have been widely used to control PB in Poland. However, during the last few years, a decrease in efficacy of chemical protection against PB was reported from many regions. Bioassays for resistance monitoring of PB to pyrethroid, chloronicotinyl and organophosphorous insecticides were performed in the Institute of Plant Protection, Poznań before the year 2004. Results demonstrated some level of resistance to alpha-cypermethrin, cypermethrin, deltamethrine, lambda-cyhalothrin, esfenvalerate, and also acetamiprid (Węgorek, 2005). The results indicated that populations tolerant to pyrethroids and acetamiprid were not cross-resistant to chloropiryfos. The widespread use of pyrethroids in Poland could lead to control failure. It creates the need for constant monitoring of PB resistance and for further studies on resistance mechanisms.

Key words: Pollen beetle, insecticide resistance, pyrethroids, organophosphorous, chloronicotinyls

Introduction

Oilseed rape is cultivated on about 600 000 ha (2005) in Poland but the total yield is low: ~1 000 000 t. The Pollen beetle (PB) is the most serious pest of oilseed rape in Poland. Pyrethroids, organophosphorous and chloronicotinyls have been widely used insecticides to control PB in Poland. The cost of chemical protection of oilseed rape fields against PB in Poland totals on average 40 €/ha; 30 million €/ha on a global scale. In spite of insecticide applications, yield losses resulting from PB feeding and insecticide resistance are high and amount on average 15-25%. Over the past 50 years there has been a constant and strong selective pressure of insecticides on Polish PB populations and this is a factor which accelerates the process of increasing resistance of this species. Over a period of 27 years pyrethroids (deltamethrin, cypermethrin, alpha-cypermethrin, zeta-cypermethrin, lambda-cyhalothrin, esfenvalerate, ethofenprox) have been most commonly used for controlling PB in Poland. During the last few years a decrease of efficacy of this group of insecticides was reported from many regions of Poland. Risk of increase of tolerance to acetamiprid and pyrethroids has created a need for resistance monitoring and formulating strategies for the management of PB resistance to all synthetic insecticides (EPPO, 1999; EU, 1991).

Bioassays of selected pyrethroids, chloronicotinyls and organophosphorous insecticides for resistance monitoring in PB were performed in the Institute of Plant Protection in Poznań prior to 2004. Continuation of this work by Scientific Project 2P06R 09228 has been supported by Polish Ministry of Scientific Research and Information Technology since the year 2005.

Materials and methods

Laboratory tests

In laboratory tests the standard method recommended by Insecticide Resistance Action Committee (IRAC method nr.7) was used. Three insect populations originating from western Poland: Winna Gora, Skoki and Nowy Tomysl were selected for testing. A representative sample of PB in selected field populations and sufficient non-infested, untreated inflorescences and leaves were collected for testing. Chemicals used were:

Pyrethroids:

- alpha-cypermethrin (Fastac 100 EC) 25, 33, 50 ppm (parts per million) concentrations were tested (recommended concentration in Poland: 25-50 ppm)
- deltamethrin (Decis 2,5 EC) 25, 33, 50 ppm concentrations were tested (recommended concentration in Poland: 25-50 ppm)
- cypermethrin (Sherpa 100 EC) 100, 150, 200 ppm concentrations were tested (recommended concentration in Poland: 100-200 ppm)
- esfenvalerate (Sumi–Alpha 050 EC) 50, 80, 125 ppm concentrations were tested (recommended concentration in Poland: 47 -125 ppm)
- lambda–cyhalothrin (Karate Zeon 050 CS) 20, 40, 80 ppm concentrations were tested (recommended concentration in Poland: 25-62.5 ppm)

Chloronicotinyls:

- acetamprid (Mospilan 20 SP) 50, 66, 100 ppm concentration were tested (recommended concentration in Poland: 50-100 ppm)

Organophosphorous + pyrethroid:

- chlorpyrifos + cypermethrin (Nurelle D 550 SC) 500, and 1000 ppm concentrations of chorpyrifos were tested (recommended concentration in Poland: 500-1500 ppm)

Accurate dilutions of tested active substances from commercially-available products were used in the selected doses expressed in parts per million (ppm). Rape inflorescences and leaves were dipped in the insecticides at the various test concentrations for about five seconds then placed on a paper towel to dry. Controls were dipped in water. Untreated and treated dry plant material was placed into 0.9 l jars with 10 cm diameter filter paper lining the base and 100 PB were placed in each jar. Three replicates were conducted for each concentration and the control.

Lethal effects of the active ingredient of the insecticides was determined after 120 hours of application and expressed as percent mortality of PB at each dose, relating to untreated (control) mortalities using Abbott's formula (Abbot, 1925) if needed. Doses were expressed at ppm.

At each assessment, beetles were classed as either: (a) unaffected, giving a normal response (such as taking a co-ordinated step) when gently stimulated by touch, or (b) dead or affected, giving an abnormal response to stimulation. Corrected Mortality = 100 x (P-C/100-C) where P = % mortality in treatment, C = % mortality in controls. Tests were performed in laboratory conditions: 22-24° C and photoperiod of 16:8 (L:D).

Results and discussion

Results (Table 1) demonstrated some level of resistance of PB to alpha-cypermethrin, deltamethrin, lambda-cyhalothrin esfenvalerate, cypermethrin and also to acetamiprid. In laboratory studies (2004 and 2005) pyrethroid insecticides and acetamiprid were least

effective in controlling PB. Survival at the recommended concentration in the case of alpha-cypermethrin, deltamethrin, lambda-cyhalothrin, esfenvalerate and also in the case of acetamiprid indicated occurrence of resistance in the populations tested. Organophosphorous insecticides showed very high toxicity to PB.

The results indicated that populations tolerant to pyrethroids and acetamiprid were not cross-resistant to chloropyrifos.

The widespread use of pyrethroids in Poland can lead to control failure. Understanding the conditions which favour the causes and development of the resistance as well as the mechanisms of resistance are the crucial challenges for the future.

The pollen beetle is an example of a pest insect species that can develop strong resistance mechanisms to some active substances used to control it (Łąkocy, 1967, 1973; Hansen, 2003). Problems with PB resistance occur also in Germany, Sweden, Denmark and Estonia. Also in our study, populations from all three areas in Poland demonstrated a high level of resistance to the pyrethroid group, and some level of resistance to chloronicotinyls group. The resistance mechanism of surviving beetles is not known, although some symptoms point at an oxidative mechanism, However, this requires further research.

Table 1. Susceptibility of pollen beetle populations from three regions in Poland to insecticides in the 2004 and 2005 season.

Susceptibility to alpha-cypermethrin			
population	**% mortality at 25 ppm 2004/2005**	**% mortality at 33 ppm 2004/2005**	**% mortality at 50 ppm 2004/2005**
Winna Góra	25/37	38/43	45/50
Skoki	15/20	25/30	30/32
Nowy Tomyśl	10/40	20/38	40/46

Susceptibility to deltamethrin			
population	**% mortality at 25 ppm 2004/2005**	**% mortality at 33 ppm 2004/2005**	**% mortality at 50 ppm 2004/2005**
Winna Góra	20/40	25/40	40/60
Skoki	40/60	25/35	45/50
Nowy Tomyśl	20/25	25/50	40/60

Susceptibility to cypermethrin			
population	**% mortality at 100 ppm 2004/2005**	**% mortality at 150 ppm 2004/2005**	**% mortality at 200 ppm 2004/2005**
Winna Góra	30/50	40/60	45/65
Skoki	40/65	50/50	55/65
Nowy Tomyśl	30/50	30/60	40/60

Susceptibility to esfenvalerate			
population	**% mortality at 50 ppm 2004/2005**	**% mortality at 80 ppm 2004/2005**	**% mortality at 125 ppm 2004/2005**
Winna Góra	40/48	55/58	60/57
Skoki	38/45	55/52	64/57
Nowy Tomyśl	43/35	50/47	62/60

Susceptibility to lambda-cyhalothrin			
population	**% mortality at 20 ppm 2004/2005**	**% mortality at 40 ppm 2004/2005**	**% mortality at 80 ppm 2004/2005**
Winna Góra	30/28	35/42	65/62
Skoki	32/35	30/38	72/70
Nowy Tomyśl	38/35	44/37	70/75

Susceptibility to acetamiprid			
population	**% mortality at 50 ppm 2004/2005**	**% mortality at 66 ppm 2004/2005**	**% mortality at 100 ppm 2004/2005**
Winna Góra	78/85	90/87	95/95
Skoki	70/67	88/94	93/95
Nowy Tomyśl	75/80	85/90	90/84

Susceptibility to chloropyrifos+cypermethrin		
population	**% mortality at 500 ppm 2004/2005**	**% mortality at 1000 ppm 2004/2005**
Winna Góra	100/100	100/100
Skoki	100/100	100/100
Nowy Tomyśl	99/100	100/100

Conclusions

The constant monitoring of PB susceptibility levels to insecticides used in Poland and studies on mechanisms of PB resistance to them will help the formulation of the best strategy for managing PB resistance. At present, the general principles of a strategy involve the rational application of all recommended insecticides and their rotation according to the different modes of their toxicity.

Poland is now a member of the European Union (since May 2004). In August 2004, the Polish Ministry of Agriculture changed some directives concerning research on the effectiveness of plant protection products (Dziennik Ustaw, 2004). Presently, the registration authorities must take into consideration the resistance risk analysis based on EPPO standards and recommendations (EPPO, 2001). So during registrative investigations, resistance phenomenon of agrophages must be seriously taken into consideration. Also chemical companies should take responsibility for constant efficacy controls of their products and update them if needed in accordance with new results from susceptibility monitoring. The future of chemical protection of rape plants against PB in Poland will be dependent on many factors, among which the resistance of this species will be one of the most important.

References

Abbot, W.S. 1925: A method of computing the effectivenes of an insecticide. – J. Econ. Ent. 18: 265-267.

Dziennik Ustaw Nr 183. Poz.1890: Rozporzadzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 4 sierpnia 2004 r. w sprawie badań skuteczności działania środka ochrony roślin. 12716.

EPPO 2001 Standardy EPPO pp2/2-26. Good Plant Protection Practice (Dobra praktyka ochrony roślin) – OEPP/EPPO, Paryż (FR)

EPPO 1999: Guideline for the biological evaluation of plant protection products - resistance risk analysis. – Directive 99/7151: 1-32.

EU (1991) Dyrektywa Rady 91/414/ewg z dnia 15 lipca 1991 r. dotycząca wprowadzania do obrotu środków ochrony roślin. – Dziennik Urzędowy Wspólnot Europejskich L230: 1-32.

Hansen, L.M. 2003: Insecticide-resistant pollen beetles in Danish oilseed rape. – Pest Management Science 50: 1057-1059.

Łąkocy, A. 1967: Uwagi na temat odporności słodyszka rzepakowca (*Meligethes aeneus* F.) i stonki ziemniaczanej (*Leptinotarsa decemlineata* Say.) na DDT w Polsce. Prace Nauk. – IOR 9(1): 157-170.

Łąkocy, A. 1973: Teoretyczne i praktyczne aspekty odporności szkodników rolniczych na pestycydy. Prac. Nauk. – IOR 14(2): 5-84.

Węgorek, P. 2005: Preliminary data on resistance appearance of Pollen beetle PB (*Meligethes aeneus* F.) to selected pyrethroids, organophosphorous and chloronicotynyls insecticides, in 2004 year, in Poland. – Resistant Pest Management Newsletter 14(2).

Pest damage to oilseeds – Solutions (ii)

IPM – Manipulating pest behaviour

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 143-150

Responses of pollen beetles (*Meligethes aeneus*) to conspecific odours

Samantha M. Cook[1], Darren A. Murray[1,2], Nigel P. Watts[1] and Ingrid H. Williams[1]
[1] *Plant & Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK*
[2] *VSN International Ltd., 5 The Waterhouse, Waterhouse Street, Hemel Hempstead, HP1 1ES, UK*

Abstract: As part of ongoing studies to investigate insect-derived semiochemicals for use in pest control strategies for oilseed rape (*Brassica napus*), the responses of male and female pollen beetles (*Meligethes aeneus*), to conspecific odours was assessed in laboratory and semi-field experiments. In laboratory bioassays using a linear track olfactometer, we found no evidence for the existence of a male-produced aggregation pheromone; male pollen beetles did not respond to the odours from groups of other males. However, female beetles were repelled by the odours from a large group of females (200 individuals). This response was not shown in tests involving male beetles. These results suggest that females show epideictic (spacing) behaviour in response to high densities of other females. This response was found to be at least partially due to the odour of the females themselves, rather than due to the volatiles from female-damaged plant material. The results from semi-field and field-scale experiments however, failed to support preliminary laboratory evidence for the existence of a female-produced epideictic pheromone in the pollen beetle.

Key words: Aggregation, *Brassica napus*, epideictic, integrated pest management, *Meligethes aeneus*, Nitidulidae; oilseed rape, pheromone, pollen beetle, push-pull control strategy

Introduction

The pollen beetle *Meligethes aeneus* (Fabricius) (Coleoptera: Nitidulidae) is a serious pest of oilseed rape throughout Europe (Alford *et al.*, 2003). As part of a project aimed at reducing insecticide usage on oilseed rape, we are developing a 'push-pull' control strategy for this pest. Such strategies use components to deter or 'push' the pests away from the crop, while simultaneously attracting them onto trap crop areas ('pull') (Pyke *et al.*, 1987; Khan & Pickett, 2004). Turnip rape (*Brassica rapa*) shows good potential for use as a trap crop in this strategy (Hokkanen *et al.*, 1986; Cook *et al.*, 2002 b; Cook *et al.*, 2004), however, additional 'pull' components are sought in addition to finding effective repellents or deterrents that can be used as the 'push' element of the strategy.

Insect-derived semiochemicals offer possibilities for both 'push' and 'pull' components of the control strategy. Oviposition-deterring pheromones, epideictic (spacing) pheromones or alarm pheromones, if identified in the pollen beetle, could be used to 'push' this pest from the main crop, while aggregation sex pheromones could be used as 'pull' components to increase the efficiency of the trap crop. Male-produced aggregation pheromones have been identified in other Nitidulid beetles (Bartelt *et al.*, 1990; Bartelt *et al.*, 1993 a & b; Petroski *et al.*, 1994; Bartelt *et al.*, 2004), and the possibility exists that they also occur in the pollen beetle. Aggregation of beetles in the crop has been observed by several workers (Free & Williams, 1978; Nielsen & Axelsen, 1988; Buechi, 1989), however the existence of an aggregation pheromone has been little-studied (Bartlet & Murchie, unpublished data). Likewise, epideictic responses of female pollen beetles have been demonstrated (Ruther & Thiemann, 1997) but no recent studies have been made to exploit this behaviour in pest control strategies.

We investigated the olfactory responses of male and female pollen beetles to conspecific odours in three experiments conducted at increasingly-realistic spatial scales (laboratory, semi-field arena and field-scale); the periodicity of response was also investigated.

Material and methods

Laboratory bioassays

The responses of male and female beetles to the odours of conspecifics were assessed in a series of dual choice test bioassays using a linear track olfactometer (Cook *et al.*, 2002 a). Beetles were collected using a sweep-net from oilseed rape crops on Rothamsted Farm. Their sexes were determined (Cook *et al.*, 2002 a; Cook *et al.*, this issue) and they were maintained in single-sex groups in ventillated plastic boxes containing oilseed rape racemes until required for experiments. Responses of individual beetles to experimental stimuli were tested. Five beetles of the same sex were placed in the holding pot of the olfactometer and the response of the first beetle that emerged to make a choice between the two experimental odours was recorded. The replicate was stopped immediately after this: the beetles were removed and the horizontal wire in the olfactometer was replaced with a clean one. New beetles were introduced and the procedure conducted five times after which, the olfactometer was cleaned. Two olfactometers were used and the test odours were introduced into both chambers (chamber 1 and chamber 2) in both directions (left and right) of both olfactometers (olfactometer 1 and olfactometer 2) an equal number of times to reduce bias. Eighty individuals were tested in each experiment. Oilseed rape racemes were excised from glasshouse-grown plants. Each Beetle was used only once and all experimental material was changed every 4 h. Four experiments were carried out for males and seven experiments carried out for females as detailed in Figure 2. In each test, 10 g oilseed rape material was used. In experiment 7, racemes were excised from plants upon which 200 female pollen beetles had been maintained for 6 h (the beetles were removed prior to the test) and undamaged plants (without beetles), respectively.

In each experiment, the proportion of beetles responding to each odour was analysed by a Binomial exact test against 50% (GenStat release 8.1, VSN International, Hemel Hempstead, UK).

Semi-field arena bioassays

This experiment was designed to investigate, in more natural conditions, the existence of epideictic behaviour of female pollen beetles and the periodicity of any such responses. A polytunnel bioassay adapted from Cook *et al.* (2002 b) was used. Five oilseed rape plants (cv. Aries) each with 50% flowering on the main raceme (growth stage 65 of the BBCH scale, Lancashire *et al.*, 1991) were placed in a line across the polytunnel with 1 m between each plant. A bait was suspended from a cane, which was the same height as the main raceme, positioned just behind it). Baits comprised 5 g oilseed rape racemes with stems wrapped in damp cotton wool and covered in silver foil, with few (25), many (200) or no pollen beetles: 25 female pollen beetles, 25 male pollen beetles, 200 female pollen beetles, 200 male pollen beetles, control (5 g OSR) confined within a small, fine-mesh bag. Pollen beetles (1,000 of undetermined sex) were released 5 m down wind from the plants and the number of beetles on each plant was recorded after 8 h. All the beetles found on each plant were placed in labelled tubes and stored at -20 °C until their sexes could be determined.

The experiment was conducted as a Latin square design, with each treatment occupying each position in the polytunnel once during the experiment (5 days in total). Plants were changed daily, and pollen beetles were used only once (both those released, and those used as bait). The experiment was repeated three times using beetles at different periods in their life

cycle: over-wintered beetles just after their main emigration onto the crop (mid April), beetles in their mid reproductive season (early June), and new generation beetles (late July).

The proportions of female beetles found on each treatment were logit-transformed and analyzed by analysis of variance (GenStat, as above).

Field bioassays

This experiment aimed to determine if epideictic effects could be demonstrated in the field. The periodicity of response was also investigated. Green water traps baited with five different treatments were placed in a crop of oilseed rape in a Latin square design. Treatments were as follows:

1. Few females (10 female pollen beetles maintained on 5 g oilseed rape)
2. Many females (100 female pollen beetles maintained on 5 g oilseed rape)
3. Few males (10 male pollen beetles maintained on 5 g oilseed rape)
4. Many males (100 male pollen beetles maintained on 5 g oilseed rape)
5. Control (5 g oilseed rape flowers)

Oilseed rape racemes were excised from glasshouse-grown plants and their stems were wrapped in damp cotton wool and silverfoil as described above. Traps were left for three days and the pollen beetles in each were counted and their sexes determined as above. The experiment was conducted on overwintered beetles in mid-April during their main migration into the oilseed rape crop, and in June, in the middle of the beetles' reproductive phase. The proportion of females caught in each treatment was analysed by ANOVA as described above.

Results

Laboratory bioassays

Male pollen beetles did not respond differently to the odour of oilseed rape or oilseed rape with 25, 50, 100 or 200 male conspecifics (Binomial exact test $P = 0.576$; $P = 0.931$, $P = 0.931$, $P = 0.738$, respectively; see Figure 1). These results indicate that male beetles do not show aggregation behaviour in response to volatiles of other males.

The responses of female pollen beetles varied with experimental stimuli. More females orientated towards the odour of oilseed rape with 25 females than the oilseed rape control, although the difference was not significant (Binomial exact $P = 0.093$; Figure 2). Females tended to orient away from the odour of oilseed rape with groups of females greater than 25, although this difference was only significant when the group comprised 200 females (Binomial exact $P = 0.057$, $P = 0.146$ and $P < 0.001$ for groups of 50, 100 and 200 females, respectively; Figure 2). Females did not orient away from the odour of oilseed rape with a group of 200 male beetles (Binomial exact $P = 0.738$; Figure 2), indicating that the 'repellent' odour was female-specific. The repellent odour could be due either to the beetles themselves, or to the plant volatiles released upon damage specific to females (e.g. oviposition damage). Females did not respond differently to undamaged oilseed rape odours or those damaged by 200 females prior to the test (Binomial exact test $P = 0.931$; Figure 2). However, females did orient away from the odour of 200 females without oilseed rape when tested against a blank-air control (Binomial exact test $P = 0.033$; Figure 2).

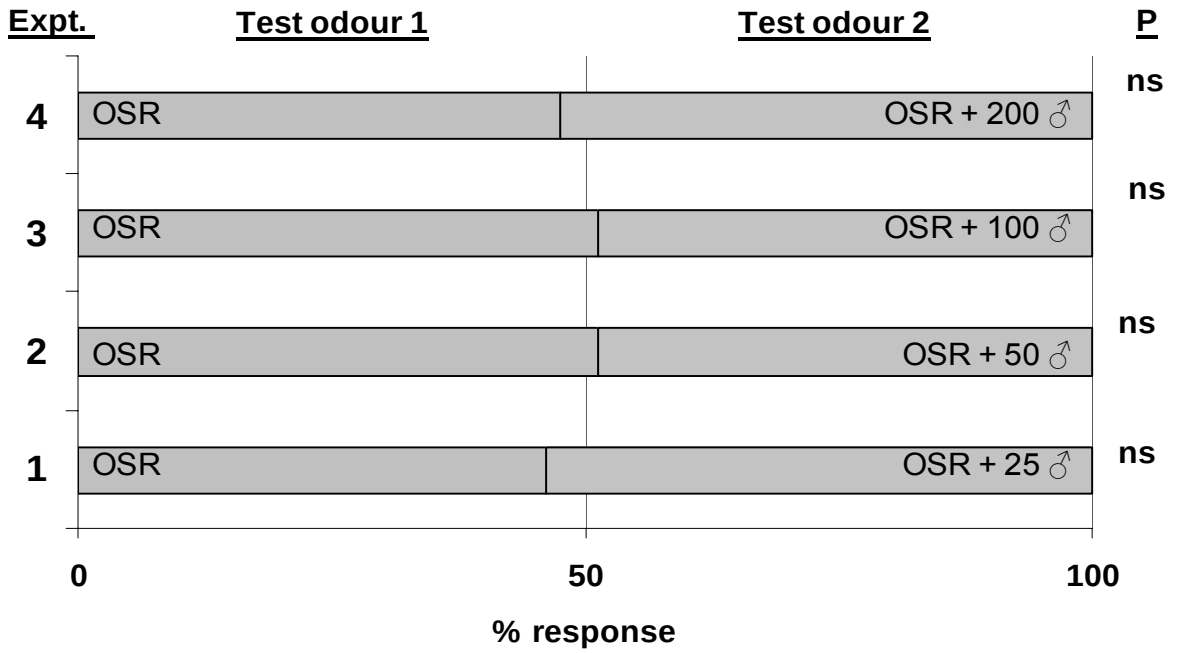


Figure 1.Responses of male pollen beetles in a linear track olfactometer to odours of oilseed rape (OSR) (10 g) and oilseed rape (10 g) with increasing numbers of conspecific males

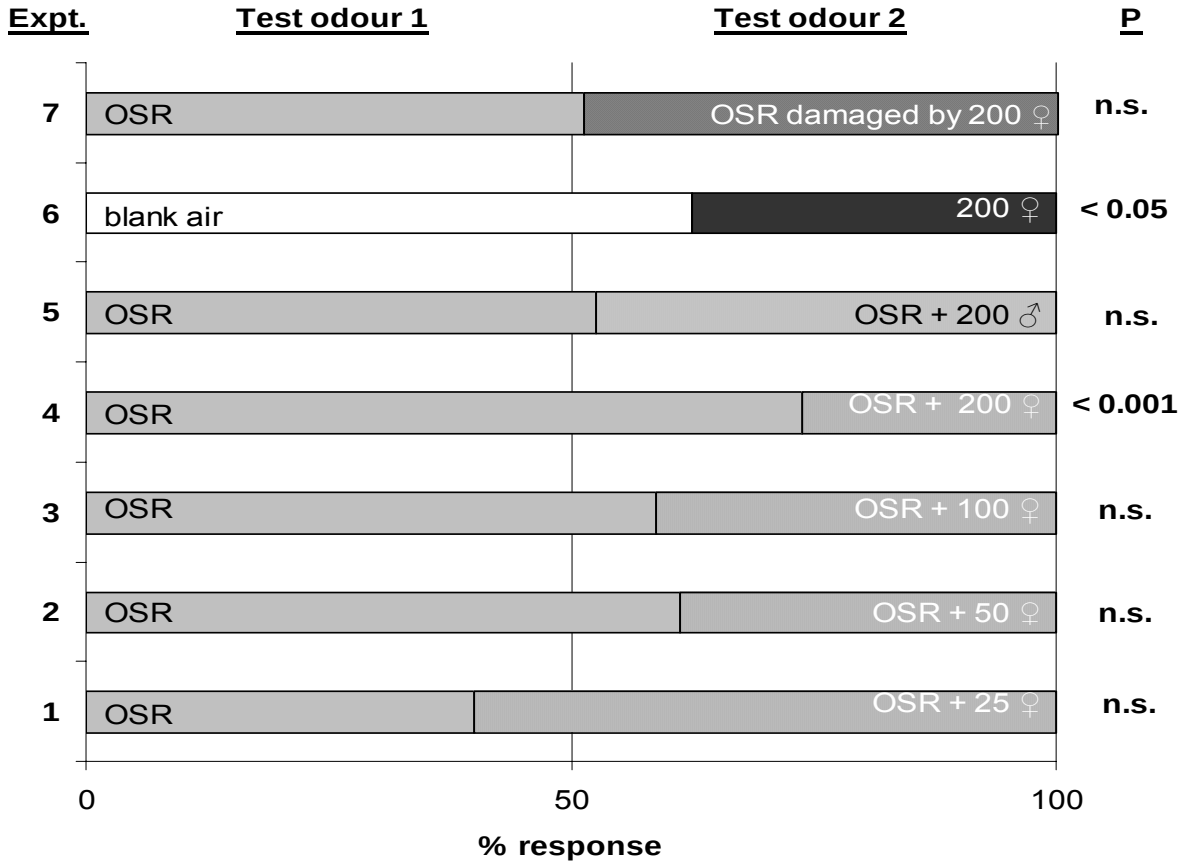


Figure 2.Responses of female pollen beetles in a linear track olfactometer to odours of oilseed rape (OSR) and conspecific beetles

Semi-field bioassays

In April, there was little variation in the mean total number of overwintered beetles colonizing plants of the five treatments (16.6, 10.6, 11.6, 15.6 and 17.2 for the control, and baits containing 25 males, 25 females, 200 males and 200 females, respectively). The proportion of beetles that were female colonizing the plants did not significantly differ between treatments (Table 1); each was similar to the proportion of females released (= 0.49, estimated from other beetles from the same sample collected from the field).

Table 1. Mean proportion (logit-transformed, with back-transformed proportions in brackets) of female pollen beetles colonizing plants baited with few (25) or many (200) male and female pollen beetles maintained on oilseed rape racemes or a control bait (oilseed rape racemes alone) in a polytunnel bioassay, 8 h after release.

Beetles used, and time of year experiment conducted	Logit-transformed mean proportion females (back-transformed proportion)					s.e.d.	$F_{4,12}$	P
	Control	25 males	200 males	25 females	200 females			
Post-diapause, Mid-April	-0.019 (0.50)	0.0246 (0.56)	0.228 (0.56)	-0.042 (0.49)	0.11 (0.50)	0.278	0.51	0.729
Post-diapause / mid-reproductive phase, Early June	0.16 (0.54)	0.02 (0.50)	-0.07 (0.48)	-0.19 (0.45)	-0.36 (0.41)	0.339	0.69	0.616
New-generation, Late July	0.059 (0.52)	-0.078 (0.48)	0.159 (0.54)	-0.096 0.48	-0.011 0.50	0.241	0.38	0.820

In June, little variation was again seen in the mean numbers of beetles colonizing plants of each treatment (21.0, 16.8, 27.4, 25.0 and 24.0 for the control, and baits containing 25 males, 25 females, 200 males and 200 females, respectively). The proportion of females of those released was estimated at 0.46, and the proportion of beetles that were female colonizing the plants did not significantly differ between treatments (Table 1).

As with the other experiments, new generation beetles tested at the end of July did not respond differently to the treatments. The total mean number colonizing treatments were: 24.8, 30.4, 26.2, 27.0 and 31.4 on plants baited with 0 beetles, 25 males, 200 males, 25 females and 200 females, respectively. The proportion of females colonizing each plant did not differ (Table 1) and was again close to the estimated proportion of females released (= 0.45).

Field bioassays

In April little variation was seen in the mean numbers of beetles in traps of each treatment (4.0, 4.6, 6.4, 5.0 and 3.6 for the control, and baits containing 10 males, 10 females, 100 males and 100 females, respectively). In June, again little variation was seen in the mean numbers of beetles in traps of each treatment (20.0, 7.6, 9.4, 8.0 and 10.8 for the control, and baits containing 10 males, 10 females, 100 males and 100 females, respectively). In both April and June, the lowest proportion of females found were in traps baited with the highest number of females. However, the proportion of females caught did not differ significantly between

treatments in either case (Table 2). The experiment was not repeated on new generation beetles, as these were unlikely to show epideictic responses since they are not sexually active at this time of year.

Table 2. Mean proportion (logit-transformed, with back-transformed proportions in brackets) of female pollen beetles caught in traps baited with few (10) or many (100) male and pollen beetles maintained on oilseed rape racemes or a control (oilseed rape racemes alone) after three days in a field bioassay.

Beetles available, and time of year experiment conducted	Logit-tramsformed mean proportion females (back-transformed proportion)					s.e.d.	$F_{4,12}$	P
	Control	10 males	100 males	10 females	100 females			
Post-diapause, Mid-April	-0.13 (0.47)	-0.97 (0.27)	-1.04 (0.25)	0.73 (0.68)	-1.97 (0.12)	1.583	1.1	0.399
Post-diapause / mid-reproductive phase, June	0.69 (0.67)	0.82 (0.70)	-0.09 (0.48)	-0.56 (0.36)	0.63 (0.66)	0.674	1.58	0.244

Discussion

We found no evidence in our bioassays to support the existence of male-produced aggregation pheromones in the pollen beetle. Beetles of both sexes were not significantly more attracted to the odour of treatments contraing males than to controls in any of the three experiments in this study. Perhaps the male beetles in these experiments were not producing the pheromones as they would in the field (possibly because they were stressed by the experimental situations) or alternatively, visual cues may play a more important role in aggregation, as suggested by Free & Williams (1978). These authors noted an increased attraction of beetles to flowers with a black dot painted onto a petal to simulate a pollen beetle compared with untreated flowers (Free & Williams, 1978). This possibility merits further study.

In laboratory bioassays, we found evidence for epideictic behaviour of female beetles. Female beetles orientated away from the odour of groups of other females and the odour of 200 females was significantly repellent. The response was due to female-specific volatiles, as females were not repelled by the odour of males. The repellent volatiles were found to emanate from the females themselves, rather than to the plant material upon which the females were maintained during experiments. These results support those of Ruther & Thiemann (1997) who found that females avoided the odour of 200 other females and the odour from diethyl ether extracts of 900 unsexed beetles, but did not avoid the odour from 100 females.

Epideictic pheromones as mechanisms by which females can avoid conspecific competition for over-exploited oviposition sites could be exploited as ‘push’ components in control strategies for pests. Therefore, the epideictic responses of pollen beetles were explored further at more realistic scales and the periodicity of response was investigated. Unfortunately, the epideictic responses observed in laboratory experiments could not be clearly demonstrated at the semi-field scale; the proportion of females responding to the

treatments with many females did not differ significantly from those with few females, those with males or the control, although in the field experiments, the lowest proportions of females caught were found in traps baited with the largest number of conspecific females. As with the males, it is possible that females in these experiments were not producing the pheromone normally. Further investigations are needed to identify the female-specific volatiles emanating from groups of females. These compounds could then be tested in bioassays similar to those described here to test further the potential of pollen beetle epideictic pheromones in pest control strategies.

Acknowledgements

This work was funded by a Biotechnology and Biological Sciences Research Council (BBSRC) special studentship and by the UK Department for Environment, Food and Rural Affairs. Rothamsted Research receives grant-aided support from the BBSRC.

References

Alford, D.V., Nilsson, C. & Ulber, B. 2003: Insect pests of oilseed rape crops. – In: D.V. Alford (ed.): Biocontrol of oilseed rape pests, Blackwell Science, Oxford: 9-42.

Bartelt, R.J., Carlson, D.G., Vetter, R.S. & Baker, T.C. 1993a: Male-produced aggregation pheromone of *Carpophilus mutilatus* (Coleoptera: Nitidulidae). – Journal of Chemical Ecology 19: 107-118.

Bartelt, R.J., Dowd, P.F., Plattner, R.D. & Weisleder, D. 1990: Aggregation pheromone of driedfruit beetle, *Carpophilus hemipterus*: wind-tunnel bioassay and identification of two novel tetraene hydrocarbons. – Journal of Chemical Ecology 16: 1015-1039.

Bartelt, R.J., Kyhl, J.F., Ambourn, A.K., Juzwik, J. & Seybold, S.J. 2004: Male-produced aggregation pheromone of *Carpophilus sayi*, a nitidulid vector of oak wilt disease, and pheromonal comparison with *Carpophilus lugubris*. – Agricultural and Forest Entomology 6: 39-46.

Bartelt, R.J., Seaton, K.L. & Dowd, P.F. 1993b: Aggregation pheromone of *Carpophilus antiquus* (Coleoptera, Nitidulidae) and kairomonal use of *C. lugubris* pheromone by *C. antiquus*. – Journal of Chemical Ecology 19: 2203-2216.

Buechi, R. 1989: Spatial distribution of the blossom beetle *Meligethes aeneus* F. on rape and turnip rape. – Journal of Applied Entomology 108: 363-371.

Cook, S.M., Bartlet, E., Murray, D.A. & Williams, I.H. 2002a: The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. – Entomologia Experimentalis et Applicata 104: 43-50.

Cook, S.M., Smart, L.E., Potting, R.P.J., Bartlet, E., Martin, J.L., Murray, D.A., Watts, N.P. & Williams, I.H. 2002b: Turnip rape (*Brassica rapa*) as a trap crop to protect oilseed rape (*Brassica napus*) from infestation by insect pests: potential and mechanisms of action. – In: Proceedings of the BCPC Pests & Diseases conference, 18-21 November 2002, Brighton, 569-574.

Cook, S.M., Watts, N.P., Castle, L.M. & Williams, I.H.: Determining the sex of insect pests of oilseed rape for behavioural bioassays. – IOBC/wprs Bulletin 29(7): 205-211.

Cook, S.M., Watts, N.P., Hunter, F., Smart, L.E. & Williams, I.H. 2004: Effects of a turnip rape trap crop on the spatial distribution of *Meligethes aeneus* and *Ceutorhynchus assimilis* in oilseed rape. – IOBC/wprs Bulletin 27 (10): 199-206.

Free, J.B. & Williams, I.H. 1978: The responses of the pollen beetle *Meligethes aeneus*, and the seed weevil *Ceutorhynchus assimilis*, to oilseed rape, *Brassica napus* and other plants. – Journal of Applied Ecology 15: 761-774.

Hokkanen, H.M.T., Granlund, H., Husberg, G.-B. & Markkula, M. 1986: Trap crops used successfully to control *Meligethes aeneus* (Coleoptera: Nitidulidae the rape blossom beetle). – Annales Entomologici Fennici 52: 115-120.

Khan, Z.R. & Pickett, J.A. 2004: The 'push-pull' strategy for stemborer management: a case study in exploiting biodiversity and chemical ecology. – In: Gurr, Wratten and Altieri (eds.), Ecological Engineering for Pest Management: Advances in habitat manipulation for arthropods. CAB International, Wallingford.: 155-164.

Lancashire, P.D., Bleiholder, H., Vandenboom, T., Langeluddeke, P., Stauss, R., Weber, E. & Witzenberger, A. 1991: A uniform decimal code for growth stages of crops and weeds. – Annals of Applied Biology 119: 561-601.

Nielsen, P.S. & Axelsen, J. 1988: Spatial distribution of the pollen beetle (*Meligethes aeneus* F.) in spring sown rape (*Brassica napus* L.) in Denmark, and its importance for sampling. – Journal of Applied Entomology 105: 35-40.

Petroski, R.J., Bartelt, R.J. & Vetter, R.S. 1994: Male-produced aggregation pheromone of *Carpophilus obsoletus* (Coleoptera: Nitidulidae). – Journal of Chemical Ecology 20: 1483-1493.

Pyke, B., Rice, M., Sabine, B. & Zalucki, M.P. 1987: The push-pull strategy - the behavioural control of *Heliothis*. – Australian Cotton Grower, May-July: 7-9.

Ruther, J. & Thiemann, K. 1997: Response of the pollen beetle *Meligethes aeneus* to volatiles emitted by intact plants and conspecifics. – Entomologia Experimentalis et Applicata 84: 183-188.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 151-158

Responses of pollen beetles (*Meligethes aeneus*) to petal colour

Samantha M. Cook, Matthew P. Skellern, Matthew J. Smith and Ingrid H. Williams
Plant & Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK.

Abstract: It is well known that the pollen beetle (*Meligethes aeneus*), a major pest of oilseed rape (*Brassica napus*) inflorescences, is attracted to the yellow colour of the flowers. Little is known however, about how variation in flower colour might affect host plant location and subsequent infestation by this pest. We investigated the responses of new-generation pollen beetles to a range of differently-coloured traps and to flowers of a white-petalled oilseed rape cultivar that had been dyed different colours. In the field, beetles were significantly more attracted to sticky traps coloured yellow than other colours. Black and blue coloured traps caught fewest beetles. In wind tunnel and field experiments using racemes and potted plants with dyed flowers, flowers dyed blue were less attractive than yellow, untreated white, and red flowers. The potential for manipulation of petal colour in control strategies for this pest is discussed.

Key words *Brassica napus*, colour, integrated pest management, *Meligethes aeneus*, oilseed rape, pollen beetle, push-pull, trap crop, visual cues

Introduction

The pollen beetle, *Meligethes aeneus* (Fabricius) (Coleoptera: Nitidulidae), is an important pest of both winter and, in particular, spring oilseed rape (*Brassica napus*) throughout Europe. It is univoltine. On emergence from overwintering sites, adult beetles feed initially on pollen from spring-flowering plants of several different families, before migrating onto brassicaceous species, including oilseed rape, to reproduce. The new-generation adults feed on pollen from many autumn-flowering plant species before overwintering in leaf litter.

Economic losses are caused by both the adults and the larvae through the destruction of the buds and flowers, reducing yield potential (Nilsson, 1987). Pyrethroid insecticides have conventionally been used to control pollen beetles and other pests of oilseed rape, but insecticide resistance has been reported (Hansen, 2003; Heimbach *et al.*, this issue; Wegorek, this issue), and alternative integrated pest management strategies are sought. As such, a 'push-pull' strategy (Pyke *et al.*, 1987; Khan and Pickett, 2004) is currently under development. The use of a turnip rape (*Brassica rapa*) trap crop as an attractive 'pull' component of this strategy is under investigation (Cook *et al.*, 2002b), and a greater understanding of the role of colour in host location for the pollen beetle has potential in the further development of this strategy.

Pollen beetles employ both visual and olfactory cues in the location of their host plants for feeding and oviposition. Several studies have demonstrated the attraction of pollen beetles to host plant volatiles over short distances (e.g. Cook *et al.*, 2002a; Mauchline *et al.*, 2005). Whilst oilseed rape leaf and flower extracts have also been demonstrated to be attractive from greater distances of at least 20 metres in the field (Evans and Allen-Williams, 1994), visual cues may play an important role over an even larger scale.

The attractiveness of the colour yellow to pollen beetles has been widely documented. Wasmann (1926) first noted that pollen beetles were attracted to "rape yellow". More recent studies have also reported a greater attraction to yellow than to other colours (Laska *et al.*,

1986; Buechi, 1990). In studies investigating the effects of five different colours on pollen beetle catches in unbaited petri-dish water traps, yellow was found to be most attractive, trapping at least 71% of the total beetles caught. Some attraction was also noted to yellow-green, but responses to white and grass-green traps were found to be lower and variable. Cream and black traps were unattractive (Blight & Smart, 1999). Finch (1991) observed a reduction in the number of pollen beetles caught in water traps painted part yellow and part black when compared with all-yellow traps.

Advances in new plant breeding techniques offer potential for the development of new varieties of oilseed rape with altered petal colour, which could be far less visually attractive to pollen beetles than current yellow-petalled varieties. Yellow-petalled lines of oilseed rape have been found to be more attractive to pollen beetles than lines of cream flower colour, which in turn were more attractive than white-flowered lines (Giamoustaris & Mithen, 1996), but no studies have yet examined the responses of pollen beetles to a wider range of petal colours. In the current study, a series of three preliminary experiments were conducted to investigate pollen beetle responses to colour; to explore how wider variation in flower colour might affect host plant location and subsequent infestation by pollen beetles.

Materials and methods

Dyed plant material and pollen beetles

Experiments were conducted at Rothamsted Research, Harpenden, UK during July and August 2004. In Experiments 2 – 3, a white-flowered experimental variety of oilseed rape (F4 H010191-S5-2, CPB Twyford, Cambs., UK) was used to provide a neutral base petal colour, which could easily be manipulated using dyes taken up by the plants, manifesting as an alteration in the colour of the petals. Preliminary investigations showed that food colourings rather than clothing dyes were most suitable for this purpose, as they tended to be readily taken up by the plants, yet exhibited less phytotoxicity. Plants were glasshouse-grown, individually in 3 l pots, and were used in experiments at early to full flowering stage (BBCH growth stage 63-65, Lancashire *et al.*, 1991). Either whole plants or excised racemes were dyed. In Experiment 2, excised racemes were dyed yellow, blue or red, by placing them in a 30% dye solution in water for one hour (colourings manufactured by Supercook, Sherburn-in-Elmet, Leeds, UK). Racemes of an untreated control (white) were placed in water for the same duration. In Experiment 3, whole plants were treated: the roots were gently washed to remove as much compost as possible before being placed in a 50% solution of yellow, red or blue food colouring (as above) in water. In this case, as a much greater amount of plant material was involved, the plants were given six hours to take up the dye.

Pollen beetles for use in wind tunnel experiments (Experiment 2) were collected from oilseed rape crops using a sweep net. They were kept at 10°C with a 15.5L:8.5D photoperiod in ventilated plastic boxes, with flowering oilseed rape racemes provided as a food source, before use in the experiments. All other experiments were conducted in the field, where the plants were colonised by natural populations of pollen beetles.

Experiment 1: Response of pollen beetles to coloured sticky traps in the field

Sticky traps were made from red, blue, yellow, green, black or white rectangular pieces of card (98.6 mm x 210 mm) coated with Oecotak (Oecos Ltd, Kimpton, Hertfordshire, UK). The sticky traps were set out in a 10 m x 10 m grid within a spring oilseed rape crop, according to a 6 x 6 Latin square design, where each colour occurred once within each row and column. Traps were spaced 2 m apart along both rows and columns. Holes made in the top and bottom of the cards enabled them to be threaded onto 1 m fibreglass flexicanes. The traps angled at 45° to the ground as other studies have shown this is most efficient for trapping pollen beetles

(Blight & Smart, 1999). A vertical cane, taped to the top of each angled cane provided support. The height of the sticky traps was adjusted, by sliding them along the angled canes, to 25 cm above the ground. After 6 h, the traps were removed and the numbers of pollen beetles caught on the cards were assessed.

Experiment 2: Response of pollen beetles to dyed racemes in a wind tunnel

The responses of pollen beetles to petal colour were investigated in the laboratory using a wind tunnel, consisting of a rectangular chamber (90 x 30 x 30 cm) through which air was pulled by an electric fan, as previously described by Du, Poppy & Powell (1996). The fan speed was adjusted to give an air flow of 10 cm/sec through the tunnel. Three racemes of each treatment (flowers dyed red, yellow, and blue, and white as an untreated control) were placed in 25 ml conical flasks containing 20 ml water. The numbers of flowers in each treatment were equalised to the lowest denominator. The four treatments were placed in a row across the wind tunnel, 50 cm downwind from the beetle release point. Fifty beetles were released, and the numbers of beetles colonising racemes of each of the treatments were assessed after one hour. Four replicates were conducted in one day, in a Latin square design such that each colour was placed in each position once within the rows each day (rows thus represented different replicates within the day, and columns represented position within the tunnel). New oilseed rape racemes and beetles were used for each replicate. The experiment was repeated over three consecutive days.

Experiment 3: Colonization of dyed whole plants in the field

Whole plants dyed yellow, red and blue, and white-petalled untreated plants were set out in a 4 x 4 Latin square configuration, where each treatment occurred once within each row and column. Plants were spaced 1.5 m apart. To provide support, the plants were placed in sand-filled 3 l pots, and these were half-buried in the ground. The pollen beetles colonising the plants were counted after 24 hours.

Statistical analyses

Analysis of variance (ANOVA) was used to analyse differences in the numbers of pollen beetles responding to each treatment (GenStat 7th Edition, VSN International, Hemel Hempstead, UK). For Experiment 2, data from each day were combined, and day was included in the blocking structure. Where appropriate, the data were transformed by $\log_{10}(n + 1)$, and means were compared at $P = 0.05$ using least significant difference (L.S.D.) values.

Results

Experiment 1: Response of pollen beetles to coloured sticky traps in the field

The number of pollen beetles trapped on sticky cards in the field varied significantly with colour (ANOVA $F_{5, 20} = 11.76$, $P < 0.001$; L.S.D = 3.185; Figure 1). Significantly more beetles (33% of the total trapped across all treatments) responded to yellow cards than to white, green, red, blue or black cards (22%, 15%, 14%, 10% and 6% of the total trapped, respectively; n = 229). White cards caught significantly more beetles than blue or black cards but there was no difference between the numbers of beetles responding to the white, green, and red treatments. Green cards trapped more beetles than black cards, but there was no significant difference between the numbers of beetles responding to green and blue cards. There were no significant differences between the numbers of beetles responding to the red, blue and black treatments.

In addition to the pollen beetles, the large numbers of flea beetles (*Phyllotreta* sp.) trapped on the sticky cards were also recorded (n = 3626) and differences analysed by ANOVA after transformation. There was a significant effect of colour on the numbers of flea beetles trapped ($F_{5, 20} = 17.01$, $P < 0.001$), and the responses followed a similar pattern to those of the pollen beetles. Yellow, white and green treatments caught significantly more beetles (32%, 25% and

20% of the total trapped across all treatments, respectively) than the red and blue treatments (10% and 9%), and the red and blue treatments in turn trapped more beetles than the black (5% of the total trapped).

Experiment 2: Response of pollen beetles to dyed racemes in a wind tunnel

There was a significant effect of colour on the numbers of pollen beetles colonising oilseed rape racemes in the wind tunnel ($F_{3, 24} = 4.94$, $P < 0.01$; L.S.D. = 2.084; Figure 2). Significantly more beetles were recorded on yellow, white and red racemes (32%, 28% and 28% of the total across all treatments, respectively) than on blue racemes (12% of the total recorded).

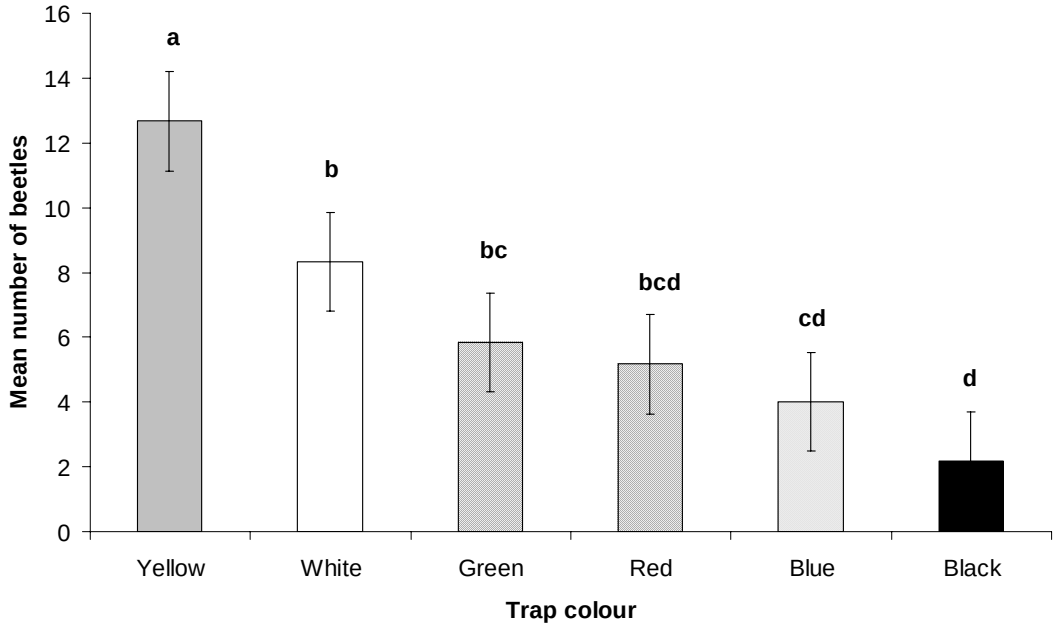


Figure 1. Mean numbers of pollen beetles (± s.e.d) trapped on coloured sticky cards in the field after 6 hours. Means showing different letters are significantly different.

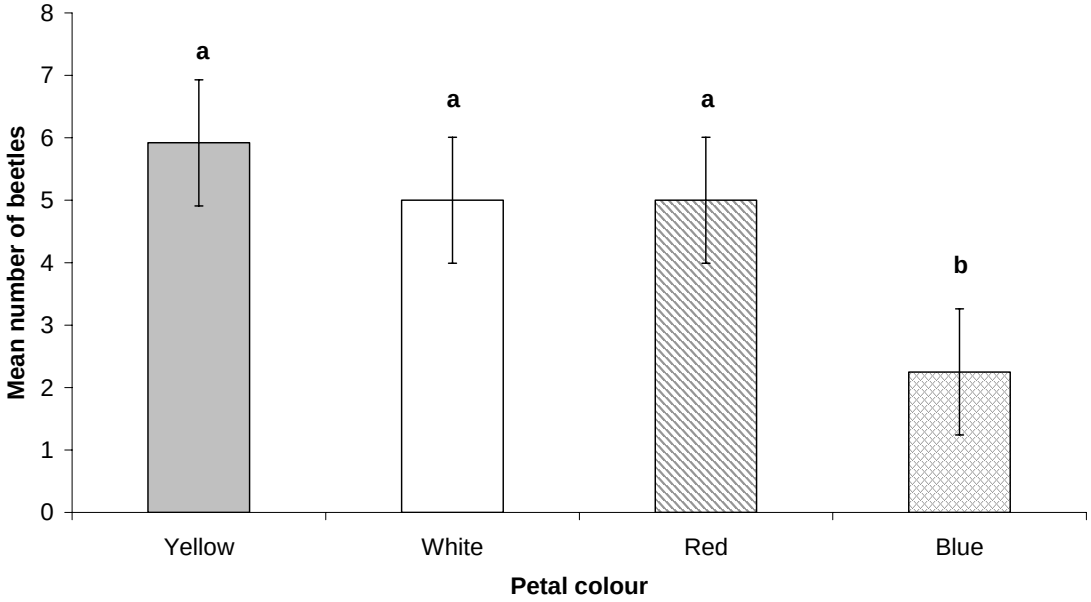


Figure 2. Mean numbers of pollen beetles (± s.e.d) colonising dyed racemes of oilseed rape in a wind tunnel, after 1 hour. Means showing different letters are significantly different.

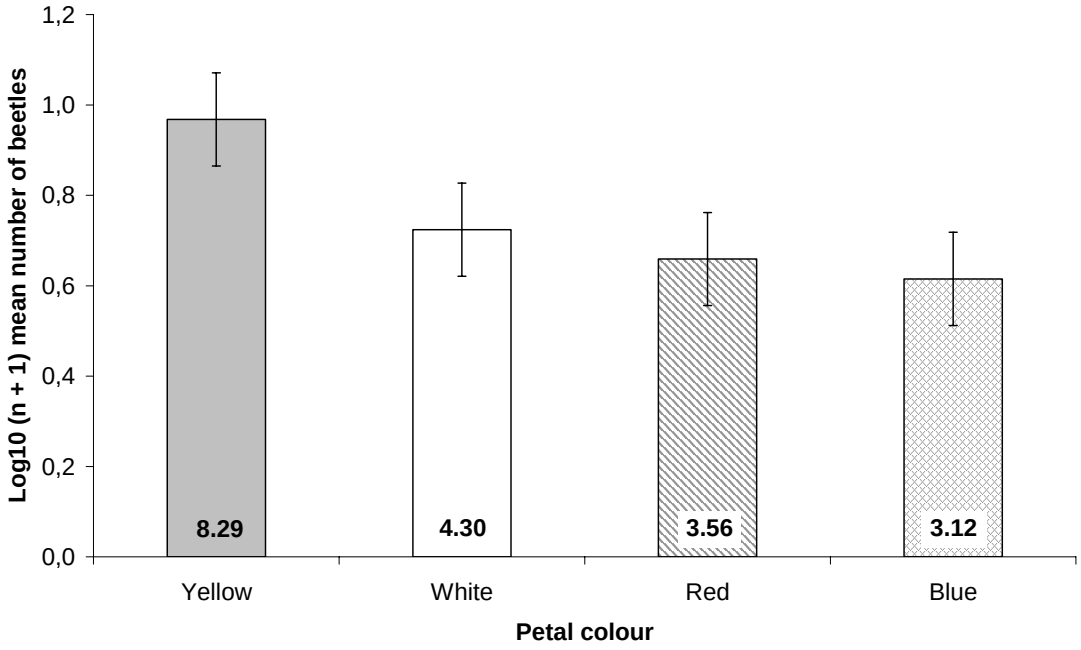


Figure 3. Mean numbers of pollen beetles (log10 (n+1) ± s.e.d) colonising whole oilseed rape plants dyed to display differing petal colours, after 24 hours. Back-transformed means are displayed at the base of each bar.

Experiment 3: Colonization of dyed whole plants in the field

The numbers of pollen beetles colonising dyed whole oilseed rape plants in the field did not vary significantly with colour ($F_{3,\ 6}$ = 4.67, P = 0.052; Figure 3). Of the total number of beetles colonising plants across all treatments, 44%, 24%, 18% and 15% were recorded on plants with yellow, white, red, and blue petals, respectively.

Discussion

The results presented here provide evidence to support the widely-held view that colour is an important visual cue used by pollen beetles during host location. In the sticky trap experiment (Experiment 1), significantly more beetles were caught on yellow cards than on cards of any other colour, suggesting that yellow is highly attractive. Fewest beetles were caught on black, blue and red cards, suggesting that these colours are relatively unattractive. These results are in line with previous studies which have shown yellow to be attractive and black to be unattractive (e.g. Finch, 1991; Blight & Smart, 1999). Numbers of pollen beetles responding to white and green cards fall between these extremes, suggesting that these colours are of intermediate attractiveness to pollen beetles. Flea beetles showed a similar pattern of response to pollen beetles in Experiment 1, and although yellow cards again trapped more beetles than other treatments, this number was not significantly different to those trapped by the white and green cards, suggesting little preference between these colours. Red and blue cards trapped significanty fewer flea beetles, suggesting that these colours were less attractive than yellow, white or green. Black cards were significantly less attractive then all other colours tested. The results reported here are similar to those of Laska *et al.* (1986), who observed that numbers of flea beetles trapped in coloured water traps did not differ significantly between yellow, white, green and red treatments, but were significantly fewer in blue traps.

Experiments 2-3 attempted to relate the variation in response of pollen beetles to different colours with their responses to floral cues using bioassays conducted at increasing spatial scales; from the wind tunnel to a field experiment. Four colours: yellow, white, red and blue, were selected from those tested in Experiment 1 for further study. The black and green treatments used in Experiment 1 were not tested in Experiments 2-3 because black food colouring was difficult to obtain, and the green dye split into its constituent blue and yellow primary components on uptake by the plant. As the sticky trap experiment had shown that there was no significant difference between the numbers of pollen beetles responding to the black, blue and red treatments, it was considered not strictly necessary to include a black treatment in later experiments.

In the windtunnel (Experiment 2), there were no significant differences between the numbers of beetles responding to the yellow, white and red racemes, but significantly fewer responded to racemes dyed blue, suggesting that blue flowers were less attractive than the other colours tested. In the field (Experiment 3), the greatest number of beetles responded to yellow-petalled plants, followed by the white control, then red and blue treatments. These differences bordered on significance, and merit further investigation since this may be explained by the small total number of beetles responding (n = 85) at the very end of the active season.

Throughout the three experiments, the basic order of colour preference is consistent (yellow > white > red > blue), but there was variation in the pattern of statistically significant differences. The distinct preference for yellow over all other colours observed in the sticky trap results was not maintained in the wind tunnel and field experiments. A possible explanation for this is that the presence of host olfactory cues reduced variation in response to visual cues. Another possibility is that the different dyes caused differences in the volatile profiles of the plants. This in turn could affect host location and colonisation by pollen beetles, thereby reducing the resolution through which colour differences can be detected. Further experiments testing the olfactory responses of beetles to the dyed plants are required before this possibility can be eliminated. Spectral reflectance measurements of the sticky traps and dyed flowers used in these experiments would enable defininition of the wavelengths to which the pollen beetles are responding.

These studies suggest that if modern plant-breeding techniques could be used to manipulate petal colour and create blue- or red-flowered lines of oilseed rape, these visually less-attractive varieties would have potential for use in integrated pest management strategies. Although studies into the implications for pollinators would be necessary, altered petal colour varieties could potentially be incorporated as a ‘push’ component into the ‘push-pull’ strategies which are currently under development.

Acknowledgements

We are very grateful to Prof. John Pickett (Rothamsted Research) for his encouragement of this work, Dr. Rosemary Collier (HRI Warwick) for discussions and ideas which led to the initiation of this study, Darren Murray (VSN International) for statistical advice and Dr. Peter Werner (CPB Twyford) for supply of the white-petalled oilseed rape. This work was supported by a Nuffield Science Foundation school’s bursary studentship (to Matthew Smith) and funded by Defra. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council.

References

Blight, M.M. & Smart, L.E. 1999: Influence of visual cues and isothiocyanate lures on capture of the pollen beetle, *Meligethes aeneus* in field traps. – Journal of Chemical Ecology. 25: 1501-1516.

Buechi, R. 1990: Investigations on the use of turnip rape as a trap plant to control oilseed rape pests. – IOBC/wprs Bulletin 13 (4): 32-39.

Cook. S.M., Bartlet, E., Murray, D.A. & Williams, I.H. 2002a: The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. – Entomologia Experimentalis et Applicata 104: 43-50.

Cook, S.M., Smart, L.E., Potting, R.J.P., Bartlet, E., Martin, J.L., Murray, D.A., Watts, N.P. & Williams, I.H. 2002b: Turnip rape (*Brassica rapa*) as a trap crop to protect oilseed rape (*Brassica napus*) from infestation by insect pests: potential and mechanisms of action. – In: Proceedings of the BCPC Pests and Diseases conference 18-21 November 2002, Brighton, 569-574.

Du, Y.J., Poppy, G.M. & Powell, W. 1996: Relative importance of semiochemicals from first and second trophic levels in host foraging behavior of *Aphidius ervi*. – Journal of Chemical Ecology 22: 1591-1605.

Evans, K.A. & Allen-Williams, L.J. 1994: Laboratory and field response of the pollen beetle, *Meligethes aeneus*, to the odour of oilseed rape. – Physiological Entomology 19: 285-290.

Finch, S. 1991: Influence of trap surface on the numbers of insects caught in water traps in brassica crops. – Entomologia Experimentalis et Applicata 59: 169-174.

Giamoustaris, A. & Mithen, R. 1996: The effect of flower colour and glucosinolates on the interaction between oilseed rape and pollen beetles. – Entomologia Experimentalis et Applicata 80: 206-208.

Hansen, L.M. 2003: Insecticide-resistant pollen beetles (*Meligethes aeneus* F) found in Danish oilseed rape (*Brassica napus* L) fields. – Pest Management Science 59: 1057-1059.

Heimbach, U., Müller, A. & Thieme, T.: First steps to analyse pyrethroid resistance of different oilseed rape pests in Germany. – IOBC/wprs Bulletin 29(7): 131-134.

Khan, Z.R. & Pickett, J.A. 2004: The 'push-pull' strategy for stemborer management: a case study in exploiting biodiversity and chemical ecology. – In: Altieri (ed.). Ecological engineering for pest management: Advances in habitat manipulation for arthropods. Wallingford, CAB International: 155-164.

Lancashire, P. D., Bleiholder, H., Vandenboom, T., Langelüddeke, P., Strauss, R., Weber, E. & Witzenberger, A. 1991: A uniform decimal code for growth stages of crops and weeds. – Annals of Applied Biology 119: 561-601.

Laska, P., Zelenkova, I. & Bicik, V. 1986: Color attraction in species of the genera *Delia* (Diptera: Anthomyidae), *Ceutorhynchus, Meligethes* and *Phyllotreta* (Coleoptera: Curculionidae, Nitidulidae, Chrysomelidae). – Acta Entomologica Bohemoslovaca 83: 418-424.

Mauchline, A.L., Osborne, J.L., Martin, A.P., Poppy, G.M. & Powell, W. 2005: The effects of non-host plant essential oil volatiles on the behaviour of the pollen beetle *Meligethes aeneus*. – Entomologia Experimentalis et Applicata 114: 181-188.

Nilsson, C. 1987: Yield losses in summer rape caused by pollen beetles *Meligethes* sp. – Swedish Journal of Agricultural Research 17: 105-112.

Pyke, B., Rice, M., Sabine, B., & Zalucki, M.P. 1987: The push-pull strategy - the behavioural control of *Heliothis*. – Australian Cotton Grower May-July: 7-9.

Wasmann, E. 1926: Versuche über den Farbensinn des Rapskäfers (*Meligethes aeneus* F.). – Zeitschrift für Wissenschaftliche Insektenbiologie 21: 147.

Wegorek, P. & Zamojska, J.: Resistance of pollen beetle (*Meligethes aeneus* F.) to pyrethroids, chloronicotinyls and organophosphorous insecticides in Poland. – IOBC/wprs Bulletin 29(7): 135-140.

Pest damage to oilseeds – Solutions (iii)

IPM – Predators and Parasitoids

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 161-163

Assessment of staphylinid beetle larvae from oilseed rape flower stands and their influence on pollen beetle larvae

Daniela S. Felsmann and Wolfgang Büchs
Federal Biological Research Centre for Agriculture and Forestry (BBA), Institute for Plant Protection in Field Crops and Grassland, Messeweg 11/12, D-38104 Braunschweig, Germany

Abstract: In Germany, large numbers of staphylinid larvae (Coleoptera: Staphylinidae) are observed together with pollen beetle (*Meligethes aeneus*) larvae in oilseed rape flower stands. From 2002-2005 the quantitative and spatio-temporal relationship of staphylinid and *Meligethes* larvae was recorded in an Integrated Crop Management (ICM) system and a standardised oilseed rape management (STN) system. In all years there were more staphylinid larvae in the ICM-system than in the STN-system. In three years the number of *Meligethes larvae* was higher in the ICM- than in the STN-system. There was a higher temporal coincidence between the larval dropping of *Meligethes* and of Staphylinidae in the ICM-system than in the STN-system in all years. In 2003, the number of pollen beetle larvae was lower in the ICM- than in the STN-system, although the STN-system was treated with insecticides. In this year, the ratio and the temporal coincidence between pests and predators seems to have been favourable in the ICM-system, so that the staphylinid larvae were even more effective in reducing the pest population than the insecticides. The predators' influence on the *Meligethes* populations can also be seen in the emergence of the new pest generation. This was lower in the ICM-system than in the STN-system in three years and is evidence of the importance of the predators in regulation of the pest populations.

Key words: Oilseed rape, Staphylinidae larvae, *Meligethes aeneus* larvae, Integrated Pest Management, predator-prey-relationship, SADIE

Introduction

Meligethes aeneus is generally considered an important pest on oilseed rape. Recently, the intensive use of insecticides has provoked resistance to pyrethroids in parts of Denmark, France and Sweden (Alford et al., 2003; Heimbach 2006). The use of natural enemies of *Meligethes* for reducing pest numbers becomes more and more important against this backdrop. Large numbers of rove beetle larvae (Coleoptera: Staphylinidae) are frequently observed together with pollen beetle larvae (*Meligethes* spp.) in oilseed rape flower stands in Germany. A predator-prey-relationship of these two groups is assumed. The spatio-temporal relationship between rove beetle larvae and *Meligethes* larvae and therefore the role of the Staphylinidae larvae in regulation of the pest population has been investigated during the MASTER (**MA**nagement **ST**rategies for **E**uropean oilseed **R**ape pests) project in two different oilseed rape management systems.

Materials and methods

The quantitative relationship of Staphylinidae larvae and *Meligethes* larvae in oilseed rape flower stands was recorded in two different oilseed rape management systems from 2002 to 2005. An ICM- (Integrated Crop Management) system with reduced tillage and without any

insecticide treatments was compared with a ploughed standardised oilseed rape management (STN-) system with insecticide treatments (pyrethroids) at BBCH stages 10, 12, 30, 33 and 60. 21 funnel traps were placed in each management system in the years 2002-2004, and in 2005 five traps were used in each system. Each trap consisted of a funnel (21 cm diameter) fixed onto a plastic bottle. The bottles were filled with 5 % sodium benzoate and sunk into the soil under the oilseed rape canopy in a small tube. The edges of the funnels were about 15 cm above soil level. The traps were emptied once a week during the growing season (BBCH 65-97). Emergence traps (0.25 m diameter) were used to monitor the emergence of the new pest generation. Ten traps were installed in each management system in 2002-2004, and five traps were used in each system in 2005.

The spatial relationship between pest larvae and Staphylinidae larvae was analysed with the computer programme SADIE (Spatial Analysis by Distance IndicEs) (Perry, 1998).

Results and discussion

In all years more (from 2-8 times as many) Staphylinidae larvae were found in the ICM-system than in the STN-system. In all years, in the week of the highest incidence of *Meligethes* larvae dropping to the ground, the number of Staphylinidae larvae caught in the traps was higher in the ICM- than in the STN-system. The number of *Meligethes larvae* was higher in 2002, 2004 and 2005 in the ICM-system than in the STN-system (twice as much up to eight times the amount). There was a higher temporal coincidence between the dropping of the pest larvae and of Staphylinidae larvae in 2002, 2003 and 2005 in the ICM- than in the STN-system. In 2004, the temporal coincidence was weak in the ICM-system but clearer than in the STN-system.

The data from 2003 were particularly interesting because there were less pest larvae in the ICM-system without any insecticide treatments than in the STN-system where insecticide treatments were applied as in common practice. In this year, the ratio (1:19) and the temporal coincidence between pests and predators seems to have been very favourable in the ICM-system, so that the Staphylinidae larvae were even more effective in influencing the pest population than the insecticides. In the other years, the ratio between pests and predators was only so favourable in the STN-system but no temporal coincidence between pests and predators was found. The analysis of the spatial relationship between Staphylinidae larvae and pollen beetle larvae showed a significant association in 2004 (SADIE-analysis: $p<0.0001$), and no significant association or dissociation between these two groups occurred in 2002 and 2003.

The influence of the predators on the pest population was also seen in the hatching of the new pest generation. Fewer *Meligethes* of the new generation emerged in the ICM- than in the STN-system in 2003 (t-test: $p<0.05$), 2004 and 2005. This seems to be a clear indication of the importance of the predators on this pest population.

It seems that there are different factors which determine the predatory influence of Staphylinidae larvae on *Meligethes larvae* in oilseed rape flower stands. As well as a temporal coincidence between the phenologies of pests and predators the number of Staphylinidae- and *Meligethes larvae*, or rather the ratio of these, seem to be very important.

Acknowledgements

This study was funded by the EU project MASTER (QLK5-CT-2001-01447).

References

Alford, D.V., Nilsson, C. & Ulber, B. 2003: Insect pests of oilseed rape crops. – In: Alford (ed.). Biocontrol of Oilseed Rape Pests, Blackwell Publishers, Oxford: 9-41.

Heimbach, U., Müller, A. & Thieme, T. 2006: First steps in analysing pyrethroid resistance of different oilseed rape pests in Germany. – IOBC/wprs Bulletin 29(7): 131-134.

Perry, J.N. 1998: Measures of spatial pattern for counts. – Ecology 79: 1008-1017.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 165-172

Oilseed rape pests and their parasitoids in Estonia

Eve Veromann, Anne Luik and Reelika Kevväi
Department of Plant Protection, Estonian Agriculture University, 64 Kreutzwaldi St. 51014, Tartu, Estonia

Abstract: The pests and their hymenopterous parasitoids present in spring and winter oilseed rape crop in Estonia were studied. *Meligethes aeneus* was abundant pest in the spring oilseed rape whereas *Ceutorhynchus assimilis* was more numerous in the winter oilseed rape. Other crucifer-specialist pests such as *Ceutorhynchus sulcicollis, C. pallidactylus, C. rapae, C. floralis* and *C. pleurostigma* were found but their abundance was very low. Also three parasitoids of *M. aeneus* larvae (*Diospilus capito, Phradis morionellus* and *P. interstitialis*) and three of *C. assimilis* larvae (*Mesopolobus morys, Stenomalina gracilis* and *Trichomalus perfectus*) were found. Larval parasitization rate of *M. aeneus* (0–7.4%) and *C. assimilis* (0–32%) was dependent on oilseed rape crop type and use of insecticides.

Key words: Oilseed rape, *Meligethes aeneus, Ceutorhynchus assimilis*, hymenopterous parasitoids.

Introduction

In Estonia, the cultivation of oilseed rape (*Brassica napus* L. and *Brassica rapa* L.) has expanded greatly in recent years and exceeded 50,400 ha in 2004 (Statistics Board, 2005). This provides a good substrate for population growth of crucifer-specialist, phytophagous pests. In Europe, the most common pests of oilseed rape are *Meligethes aeneus* Fabr., *M. viridescens* Fabr., *Ceutorhynchus assimilis* Payk., *Ceutorhynchus pallidactylus* Panz., *C. napi* Gylh., *Dasineura brassicae* Winn., *Psylliodes chrysocephala* L. and *Phyllotreta nemorum, P. undulata* and *P. diademata* (Alford *et al*., 2003).

The management of pests on the European oilseed rape crop still relies heavily on chemical pesticides, most often applied routinely and prophylactically, without regard to pest incidence (Williams, 2004). This leads to the over-use of chemical pesticides, which reduces the economic competitiveness of the crop and threatens biological diversity. Pesticides also kill natural agents of biological control that represent a resource with a great potential benefit to farmers and consumers (Alford *et al*., 1995; Williams & Murchie, 1995). By killing the natural enemies of pests, pesticide use has to be increased further to achieve pest control (Pickett *et al*., 1995; Murchie et al., 1997). More than 30 species of hymenopterous parasitoids have been reported to attack *C. assimilis* in Europe (Williams, 2003) that can provide natural control of these pests. Of these, the larval ectoparasitoid *Trichomalus perfectus* Walker (Hymenoptera: Pteromalidae) is widely distributed and particularly important (Lerin, 1987; Murchie & Williams, 1998a). In addition to killing larvae through direct parasitism, it further reduces damage to infested pods by preventing host larvae from eating their full complement of seeds (Murchie & Williams, 1998b).

Recent advances in insect management of the pests of oilseed rape have emphasised the important role of natural enemies within integrated pest management systems for the crop (Williams, 2004). The EU-funded project MASTER: MAnagement STrategies for European Rape pests aims to develop economically-viable and environmentally-acceptable crop management strategies for winter oilseed rape which maximise the biocontrol of key pests and minimise chemical inputs (Williams *et al*., 2002). This requires a much better under-

standing of pest and parasitoid taxonomy and biology throughout Europe. The pests of oilseed rape and their natural enemies have been little studied in Estonia. The present study aims to add to the understanding of rape pests and their natural enemies in Estonia and contributes to the project MASTER.

In Estonian conditions the cropping of spring oilseed rape is still prevailing but growing of winter oilseed rape is also increasing. The aim of this study was to identify key pests and their parasitoids on both winter and spring oilseed rape in Estonia and to compare the species composition on the two crops. Also, we determined the impact of insecticide use on the occurrence and phenology of oilseed rape pests and their hymenopteran parasitoids.

Materials and methods

A study with the winter oilseed rape (WOSR) cultivar *'Banjo'*, sown in August 2004 and the spring oilseed rape (SOSR) *'Mozart'*, sown in May 2005, was carried out on Pilsu Farm, Tartu County, Estonia (58°14´ latitude 26°16´ longitude). Both crops were grown with no insecticide (i0) and with insecticide (ii) cropping systems. The study site included two adjacent, approximately rectangular, 1 ha fields (two replicates for both crops). In the ii-system, the insecticide Fastac was used at green bud stage (BBCH growth stage (GS) 51 of Lancashire et al.,1991) and Karate was used at the end of flowering of the main raceme of oilseed rape plants (GS 67).

Insects were sampled with yellow water traps (210 x 310 x 90 mm). Six traps per plot were positioned at the top of the crop canopy. Traps were emptied weekly from the beginning of flowering to pre-harvest of plants (GS 80). The growth stages of the rape plants were recorded weekly (Lancashire et al., 1991; see also Meier, 2001). Traps were put out on the winter oilseed rape field on 12 May (GS 52, inflorescence emergence stage of plants) until 28 June (GS 80; pod maturation stage) and on the spring oilseed rape fields on 15 June (GS 50, green bud stage) until 10 August (GS 80). In the laboratory, the insect samples were sorted and target cruciferous specialists and their key parasitoids were identified to species and counted.

For estimation of parasitization levels, *M. aeneus* larvae were collected from oilseed rape flowers from 25 randomly chosen plants at each plot. Larvae were dissected in the laboratory. For the establishment of damage and parasitization assessments of *C. assimilis*, 25 plants were selected from each plot and all their pods were collected and incubated in emergence traps in laboratory conditions. Emerging parasitoids and larvae were counted and the percentage of damaged pods calculated.

Statistical analysis was carried out using the SAS GENMOD procedure. The weekly target of pests and parasitoids were analysed applying the Poisson distribution and the *log* link function. For the repeated measures the generalized estimation equation analysis was used. The scale parameter was estimated by Pearson's chi-square divided by the degrees of freedom if the model was overdispersed.

Results and discussion

During the study, 84 samples of WOSR and 192 of SOSR with a total of 12,918 specimens of crucifer-specialist insect pests from eight taxa were collected from the water traps (Figure 1). A more abundant crucifer-specialist community was found in the spring oilseed rape (11,177 specimens) than in the winter oilseed rape (1,742 specimens). Among them were the three major European pests: *M. aeneus*, *C. assimilis* and *C. pallidactylus*. The latter was not numerous – only a few specimens were found in winter oilseed rape. *Meligethes aeneus* was

by far the most numerous and the only one to reach true pest status. *Meligethes aeneus* emerges from hibernation sites at the beginning of May in Estonia. Therefore, in winter oilseed rape it was not very numerous, but it was very abundant in spring oilseed rape, accounting for 98.6% of the crucifer-specialist specimens caught. *Ceutorhynchus assimilis* constituted 0.9% from the total number of specialists in spring oilseed rape but 18.8% in the winter oilseed rape. A few *M. viridescens* were also caught; this species requires higher temperatures for oviposition and development compared to *M. aeneus* (Alford et al., 2003) and therefore it is not abundant in Estonia. In addition, *Ceutorhynchus sulcicollis*, *C. rapae*, *C. floralis* and *C. pleurostigma* were also captured but their abundance was low (Figure 1).

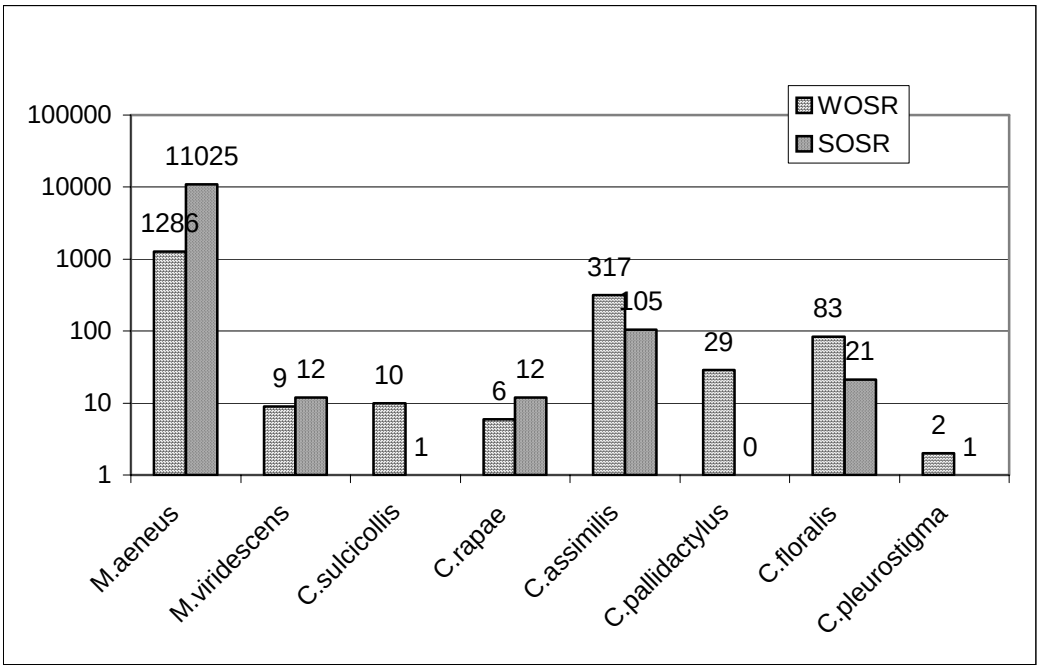


Figure 1. The species composition and the total number of crucifer-specialists caught in yellow water traps (12 per system) in winter (WOSR) and spring (SOSR) oilseed rape on Pilsu Farm, Tartu County, Estonia, in 2005.

Meligethes aeneus started to colonise the winter oilseed rape from GS 63 (30% of flowers are open) and its population peaked shortly after, on May 26 (Figure 2). In winter oilseed rape *M. aeneus* abundance was higher in the unsprayed field (i0) than in the sprayed-field (ii) throughout the study period (Figure 2; $\chi^2 = 10.27$, df = 1, P = 0.001;). Because of the late spring in 2005, *M. aeneus* oviposited in both spring and winter oilseed rape. On June 8 (GS 67–69) larvae were found and collected from flowers. Dissection of larvae showed that the parasitization rate of larvae was greater in the untreated field (8.7%) than in the sprayed field (4.8%). In an earlier study between 2002 - 2004, *M. aeneus* did not damage winter oilseed plants (Tarang, et al., 2004, Veromann, et al., 2004, 2005). In Estonia, *M. aeneus* mostly uses winter oilseed rape plants for maturation feeding and moves to the spring oilseed rape field for oviposition. The highest number of *M. aeneus* in the winter oilseed rape was at the beginning of colonisation in spring oilseed rape. In the spring oilseed rape field, the *M. aeneus* population was 6.4 times greater than in winter oilseed rape ($\chi^2 = 337.87$, df = 1, P < 0.001). *Meligethes aeneus* had two population peaks, on June 22 and August 3 in spring oilseed rape. The first peak coincided with green bud stage (GS 50) that is the most

vulnerable period for plants. Green bud stage of oilseed rape plants is the most suitable period for egg laying for *M. aeneus* (Alford, 2003). The second peak occurred during pod maturation (GS 80) and indicated emergence of the new generation from the soil. During the first peak and until the second peak, *M. aeneus* abundance was greater in the untreated field than in the insecticide-treated field, but the difference was not statistically significant. No parasitization was found in larvae from the insecticide-treated field. In the unsprayed field, the parasitization level was 3.4%. At the second peak, *M. aeneus* (new generation) abundance was greater in the treated field than in the untreated field (Figure 2, Aug 3: $\chi^2 = 4.28$, df = 1, P = 0.039; Aug 10: $\chi^2 = 21.64$, df = 1, P < 0.001), despite the application of insecticide. This difference may be explained by parasitiods, but the possibility requires additional research.

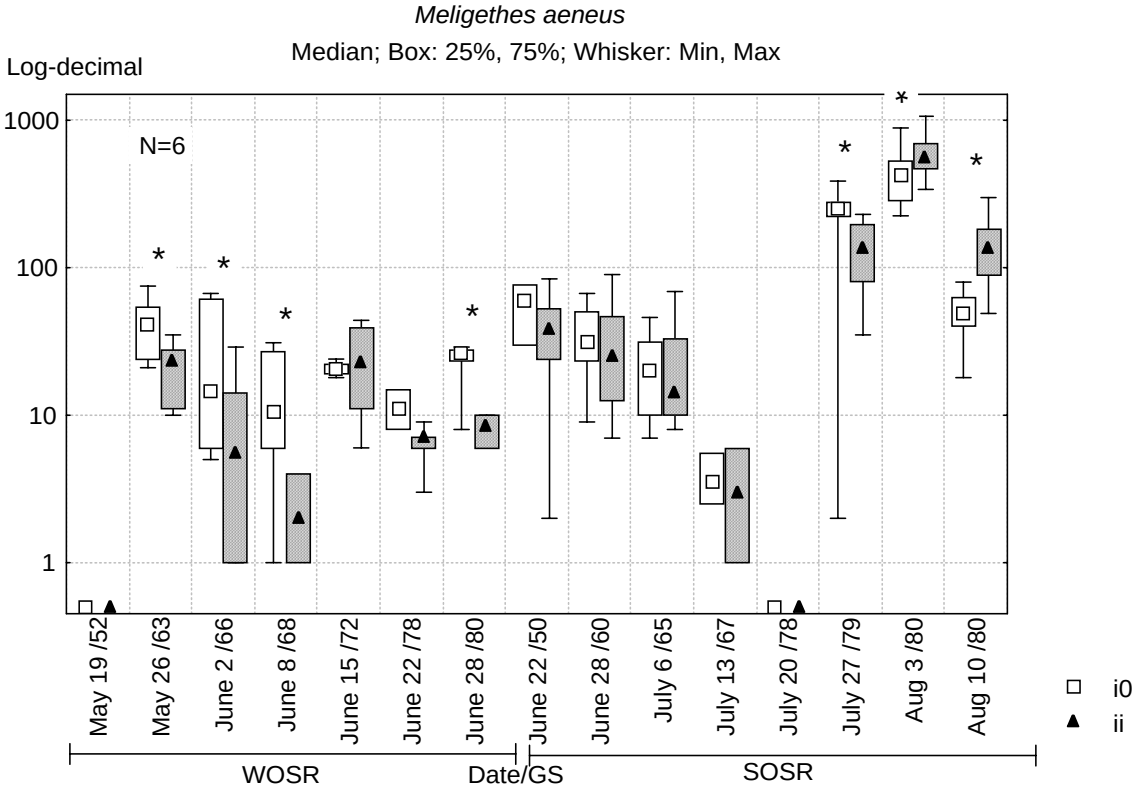


Figure 2. Abundance of *Meligethes aeneus* per yellow water trap caught at different growth stages (GS) in no-insecticide (i0) (white bars) and with insecticide (ii) (shaded bars) cropping system fields of winter (WOSR) and spring (SOSR) oilseed rape fields on the Pilsu Farm, Tartu County, Estonia, in 2005 (* indicates significant difference between treatments).

Ceutorhynchus assimilis was more numerous in the winter than the spring oilseed rape (Figures 1 & 3; $\chi^2 = 54.35$, df = 1, P < 0.001). The first *C. assimilis* were found on May 26 (GS 63 – 30% of rape plants flowers were open) and their abundance increased at pod development stage (GS 72) which is the suitable time for egg laying. This peak during pod maturation (GS 79–80) probably related to a new generation. Insecticide treatment decreased *C. assimilis* damage. In winter oilseed rape, 4.5% pods were damaged in the treated field and 14.4% in the untreated field, but larval parasitization was higher in the treated (13.6%) than in the untreated field (7.2%). In the spring oilseed rape, 0.6% and 3.7% pods were damaged in

sprayed fields and unsprayed fields, respectively. The level of larval parasitism was dependent on insecticide application. No parasitoids were found in larvae from the treated field but larval parasitization reached 32% in the unsprayed field. In spring oilseed rape, *C. assimilis* abundance was greater in the untreated than the treated field (Figure 3; $\chi^2 = 4.86$, df = 1, P = 0.027), but in both cropping systems the numbers were still lower than in the winter oilseed rape. We conclude that *C. assimilis* is phenologically better synchronised with the winter oilseed rape where reproduction mainly takes place.

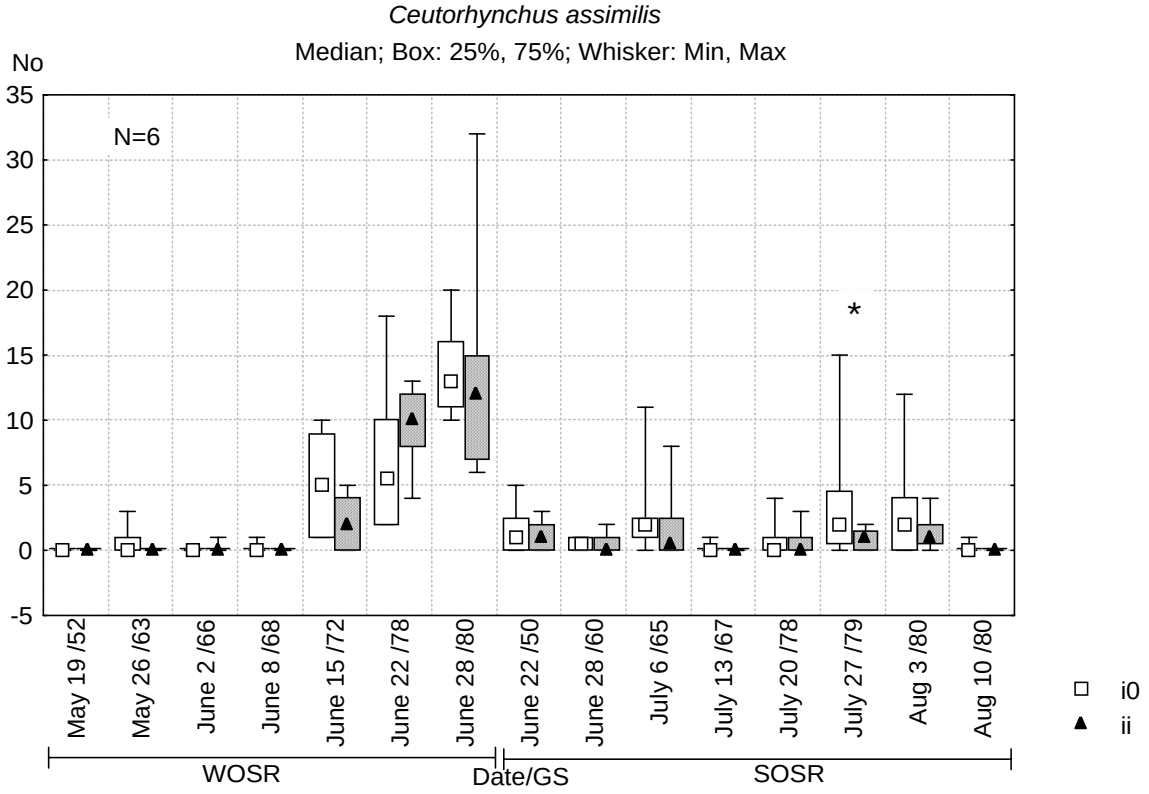


Figure 3. Abundance of *Ceutorhynchus assimilis* per yellow water trap caught at different growth stages (GS) in no-insecticide (i0) (white bars) and with insecticide (ii) (shaded bars) cropping systems of winter (WOSR) and spring (SOSR) oilseed rape fields on the Pilsu Farm, Tartu County, Estonia, in 2005 (* indicates significant difference between treatments).

We have identified three parasitoids of *M. aeneus*: *Diospilus capito, Phradis morionellus* and *P. interstitialis* and three parasitoids of *C. assimilis*: *Trichomalus perfectus, Mesopolobus morys* and *Stenomalina gracilis* in our studies (Figure 4). These parasitoid species have been found over most of Europe, from southern Sweden to France (Nilsson, 2003) and *D. capito* and *P. morionellus* parasitoids of *M. aeneus* have also been found in Finland (Hokkanen, 1989). The numbers of parasitoids of *M. aeneus* were 9.7 times greater than *C. assimilis* parasitoids. *Diospilus capito* was the most abundant parasitoid in both crops, however the numbers in the spring oilseed rape crop were 1.3 times greater than in the winter oilseed rape ($\chi^2 = 176.23$, df = 1, P < 0.0001). *Diospilus capito* is multivoltine with two or three generations in northern Europe (Billquist & Ekbom 2001; Nilsson, 2003). As the population of multivotine parasitoids in winter rape has been reported as very low (Nilsson, 2003), the

high numbers of *D. capito* in winter oilseed rape in our study was surprising. The other important *M. aeneus* parasitoids in Europe – *P. morionellus* and P. *interstitialis*, were more numerous in the winter crop. The abundance of *C. assimilis* parasitoids caught from the winter and spring oilseed rape was similar and relatively low. A total of 21 specimens of the *C. assimilis* larval ectoparasitoids: *Mesopolobus morys*, *Stenomalina gracilis*, *Trichomalus perfectus*, in the winter oilseed rape field and 28 specimens in the spring oilseed rape field were found. A total of only eight specimens of *S. gracilis*, 15 specimens of *M. morys* and 26 specimens of *T. perfectus* were found. *Trichomalus perfectus* is a univoltine ectoparasitoid whose abundance peaks 2-4 weeks after migration of *C. assimilis* into the crop. New-generation parasitoids mate on emergence and leave the crop shortly before harvest. Only females overwinter, possibly in evergreen foliage (Williams, 2003).

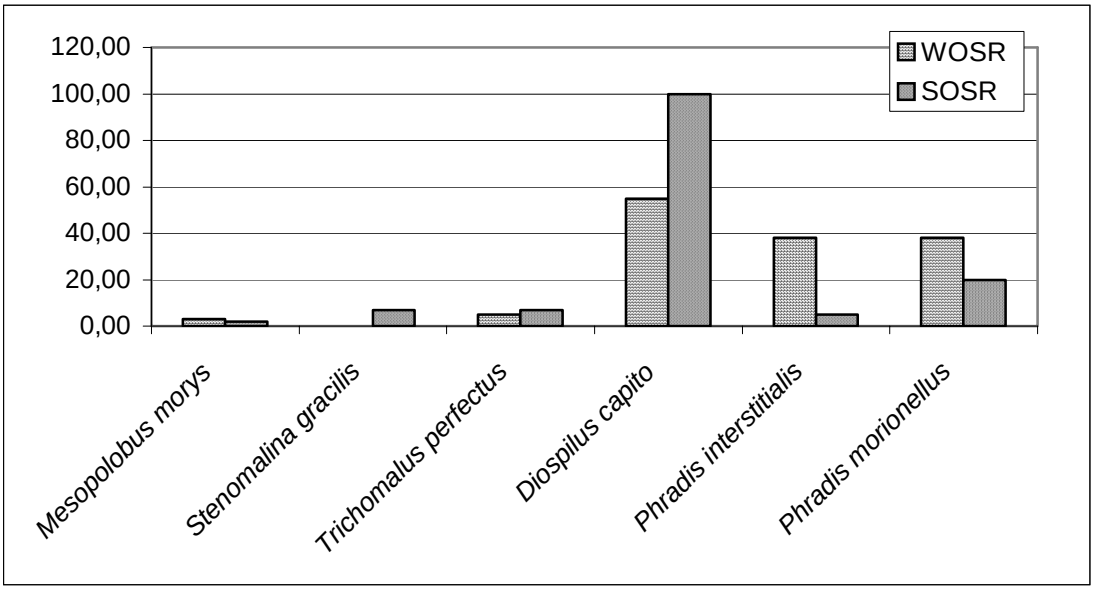


Figure 4. The species composition and the total number of key parasitoids of oilseed rape pests caught in yellow water traps (12 per system) in winter (WOSR) and spring (SOSR) oilseed rape on Pilsu Farm, Tartu County, Estonia, in 2005.

In the winter oilseed crop, the number of parasitoids started to increase at the pod development stage (GS 72) and peaked at the end of pod development (GS 78). Abundance of parasitoids in winter oilseed rape was greater in the unsprayed (i0) field than in the sprayed (ii) field (Figure 5; χ^2 =10.08, df = 1, P = 0.002). In the spring oilseed rape field, parasitoids were most numerous during pod ripening (GS 79–80). During the population peaks of the parasitoids, the dominant species in both fields was *D. capito* and their abundance was greater in the unsprayed field than in the sprayed field (χ^2 = 5.95, df = 1, P = 0.015). Parasitoid phenology was synchronised with that of their hosts. Peak abundance of *D. capito* coincided with peak abundance of *M. aeneus* (GS 80) in both fields, indicating that they probably emerged from host larvae in the oilseed rape.

We conclude that two cruciferous specialists *Ceutorhynchus assimilis* and *Meligethes aeneus* have impact for oilseed rape production in Estonia. The population of *C. assimilis* was greatest in winter oilseed rape where reproduction mainly takes place and was influenced by the parasitoids *T. perfectus, M. morys* and *S. gracilis.* Larval parasitization rate of *C. assimilis* (0–32%) was dependent on use of insecticides. The *M. aeneus* population was phonologically

better synchronised with spring oilseed rape, where numerous individuals of the new generation developed. *Meligethes aeneus* larval parasitoids *Diospilus capito, Phradis morionellus and P. interstitialis* influence the population whereas parasitization rate (0–7.4%) was dependent on insecticide treatments.

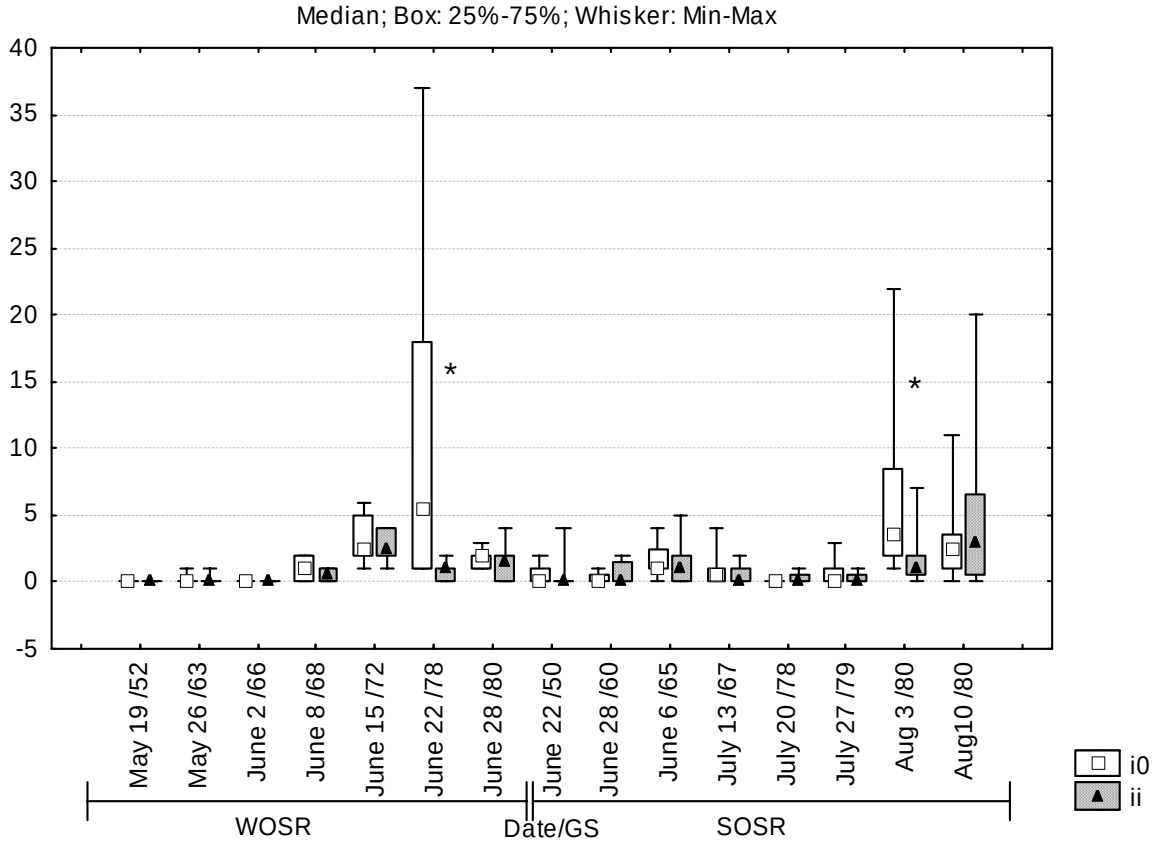


Figure 5. Abundance of key parasitoids per yellow water trap caught at different dates and growth stages (GS) in no-insecticide (i0) (white bars) and with insecticide (ii) (shaded bars) cropping systems of winter (WOSR) and spring (SOSR) oilseed rape on the Pilsu Farm, Tartu County, Estonia, in 2005 (* indicates significant difference between treatments).

Acknowledgements

We thank Marge and Madis Ajaots, Pilsu Farm, for crop husbandry. This study was supported by the EU Framework 5 project MASTER: Integrated pest management strategies incorporating bio-control for European oilseed rape pests (QLK5-CT-2001-01447), Ministry of Agriculture of Estonia and Estonian Science Foundation grants Nr 5736 and 4993.

References

Alford, D.V., Murchie, A.K. & Williams, I.H. 1995: Observations on the impact of standard insecticide treatments on *Trichomalus perfectus*, a parasitoid of seed weevil on oilseed rape in the UK. – IOBC/wprs Bulletin 18 (4): 122-126.

Alford, D.V., Nilsson, C. & Ulber, B. 2003: Insect Pest of Oilseed Rape Crops. – In: Alford, D.A. (ed.). Biocontrol of Oilseed Rape Pests, Blackwell Science Ltd, Oxford, UK: 10-41.

Billqvist, A. & Ekbom, B. 2001: Effects of host plant species on the interaction between the parasitic wasp *Diospilus capito* and pollen beetles (*Meligethes* spp.). – Agricultural and Forest Entomology 3: 147-152.

Hokkanen, H. 1989: Biological and agrotechnical control of the rape blossom beetle *Meligethes aeneus* (Col., Nitidulidae). – Acta Entomologica Fennica 53: 25-29.

Lancashire, P.D., Bleiholder, H., Boom, T.van D., Langelüddeke, P., Strauss, P., Weber, E. & Witzenberger, A. 1991: A uniform decimal code for stages of crops and weeds. – Annals of Applied Biology 119: 561-601.

Lerin, J. 1987: A short bibliographical review of *Trichomalus perfectus* Walker, a parasite of the seed pod weevil *Ceutorhynchus assimilis* Payk. – IOBC/wprs Bulletin 10: 74-78.

Meier, U. (ed.) 2001: Growth stages of mono- and dicotyledonous plants. 2nd ed. – http:// www.bba.de/veroeff/bbch/bbcheng.pdf

Murchie, A.K. & Williams, I.H. 1998a: A bibliography of the cabbage seed weevil. – IOBC/wprs Bulletin 21 (5): 163-169.

Murchie, A.K. & Williams, I.H. 1998b: Effect of host size on the sex of *Trichomalus perfectus*. – IOBC/wprs Bulletin 21 (5): 177-181.

Murchie, A.K., Williams, I.H. & Alford, D.V. 1997: Effects of commercial treatments to winter oilseed rape on parasitism of *Ceutorhynchus assimilis* Paykull (Coleoptera: Curculionidae) by *Trichomalus perfectus* (Walker) (Hymenoptera: Pteromalidae). – Crop Protection 16: 199-202.

Nilsson, C. 2003: Parasitoids of pollen beetles. – In: Alford, D.A. (ed.). Biocontrol of Oilseed Rape Pests. Blackwell Science Ltd, Oxford, UK: 73-85.

Pickett, J.A., Butt, T. M., Wallsgrove, R.M. & Williams, I.H. 1995: Minimising pesticide input in oilseed rape by exploiting natural regulatory processes. – Proceedings of the 9th International Rapeseed Congress, Cambridge, 4–7 July: 565-571.

Statistics Board 2005: Cited 3 Oct. 2005. Updated 22 July 2005. – http://pub.stat.ee/pxweb.2001/Database/Majandus/13Pellumajandus/10Taimekasvatus/10 Taimekasvatus.asp

Tarang, T., Veromann, E., Luik, A. & Williams, I. 2004: On the target entomofauna of an organic winter oilseed rape field in Estonia. – Latvijas Entomologs 41: 100-110

Veromann, E., Kevväi, R., Luik, A., Metspalu, L. & Tarang, T. 2005: Pollen beetles (*Meligethes aeneus* F) on winter and spring oilseed rape. – Agronomy 2005. Transactions of the Estonian Agricultural University.

Veromann, E., Tarang, T., Luik, A. & Metspalu, L. 2004: Pests and their natural enemies in oilseed rape in Estonia. – Latvian Journal of Agronomy 7: 12–14.

Williams, I.H. 2003: Parasitoids of cabbage seed weevil. – In: Alford, D.A. (ed.). Biocontrol of Oilseed Rape Pests. Blackwell Science Ltd, Oxford, UK: 97-112.

Williams, I.H., Büchs, W. Hokkanen, H., Johnen, A., Klukowski, Z., Luik, A., Nilsson, C. & Ulber, B. 2002: MASTER: Management Strategies for European Rape pests – a new EU Project. – The BCPC Conference 18–21 November 2002, Conference Proceedings, Brighton, UK 2: 641-646.

Williams, I.H. 2004: Advances in insect pest management of oilseed rape in Europe. – In A.R. Horowitz & I. Ishaaya (eds.): Insect Pest Management – Field and Protected Crops. Springer-Verlag, Heidelberg: 181-208.

Williams, I.H. & Murchie, A.K. 1995: The role of parasitoids. – Agronomist, Spring 1995: 13-15.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 173-179

Phenology of parasitoids (Hym., Ichneumonidae-Tersilochinae) of oilseed rape pests in northern Germany from 1995-1997

Bernd Ulber [1] and Olaf Nitzsche [2]
[1] *Department of Crop Sciences, Plant Pathology and Plant Protection Division, Entomology Section, University of Goettingen, Grisebachstrasse 6, D-37077 Goettingen, Germany*
[2] *Saxon State Agency for Agriculture, Gustav-Kuehn-Strasse 8, D-04159 Leipzig, Germany*

Abstract: The phenology of the most abundant univoltine larval parasitoids of oilseed rape pests, *Tersilochus microgaster*, *Tersilochus fulvipes*, *Tersilochus obscurator*, *Phradis interstitialis* and *Tersilochus heterocerus* (Hym., Ichneumonidae-Tersilochinae), was studied at Goettingen, northern Germany, from 1995-1997. The emergence of adult parasitoids from overwintering sites was assessed by using ground photoeclectors. Yellow water trap and Malaise trap catches were used to define the period of activity of adult parasitoids at emergence sites, as well as to monitor the time of arrival and duration of activity in current crops of oilseed rape.

The time of emergence and migration of female parasitoids to crops of oilseed rape varied between species. On many occasions, their arrival on the rape crop was found almost simultaneously with their emergence from overwintering from nearby fields. Females of *T. microgaster* were recorded from the early bud stage in March until mid-flowering. *Tersilochus fulvipes, T. obscurator* and *P. interstitialis* colonized the rape crops from mid-April onwards and adults of *T. heterocerus* occurred towards the end of April or the beginning of May. Few individuals were captured after the beginning of crop ripening. The main period of parasitoid activity coincided with the occurrence of susceptible larval instars of their hosts in the rape crop. Peak activity of all tersilochine parasitoids, except *T. microgaster*, was focussed during the flowering period of oilseed rape. Consequently, insecticide treatments during this period pose a high risk of damage to these parasitoid species. Adverse effects on parasitoid abundance may affect their impact on natural control of pest populations.

Key words: Phenology, parasitoid, oilseed rape, *Tersilochus microgaster, Tersilochus fulvipes, Tersilochus obscurator, Phradis interstitialis, Tersilochus heterocerus*

Introduction

Parasitic Hymenoptera of the subfamily Tersilochinae (Hym., Ichneumonidae) have been identified to have a major impact on insect pest populations in winter oilseed rape, particularly on Coleoptera feeding within stems and petioles as well as in buds and flowers (Jourdheuil, 1960; Nitzsche, 1998; Williams *et al*., 2004). The most abundant and effective parasitoids are *Tersilochus microgaster* (Szépligeti) (host: *Psylliodes chrysocephala), Tersilochus fulvipes* (Gravenhorst) (host: *Ceutorhynchus napi), Tersilochus obscurator* Aubert (host: *Ceutorhynchus pallidactylus*), *Phradis interstitialis* (Thomson) and *Tersilochus heterocerus* Thomson (host: *Meligethes aeneus*). The biology of these specialist solitary, univoltine larval endoparasitoids is relatively well known (Nilsson, 2003; Ulber, 2003; Ulber & Williams, 2003). Adult parasitoids overwinter in the ground of arable fields which had been grown with oilseed rape in the previous year, commonly followed by winter wheat. Like their hosts, they migrate to current oilseed rape crops from the overwintering sites in the spring. Close synchronization of the immigration flights with the appearance of susceptible hosts is considered to be essential for effective biocontrol of pest populations (Kidd & Jervis, 1996). However, comparative studies on the phenologies of tersilochine parasitoids at their emergence sites and in oilseed rape crops are still fragmentary.

The objectives of this study were to determine the period of emergence of adult parasitoids at their overwintering sites, to compare the time of emergence with time of first arrival on rape crops and to assess the periods that parasitoids are present at emergence sites as well as on crops of oilseed rape.

Materials and methods

Field studies were conducted on experimental fields at Goettingen, northern Germany, between 1995 – 1997. In this region (total area ca 100 ha), oilseed rape is grown in a three-year rotation with winter wheat and winter barley. One crop of winter wheat and oilseed rape was sampled each year. No insecticides were applied to the plots. Crop size ranged between 2.0 - 5.5 ha. Spring emergence of adult parasitoids from the ground was assessed in crops of winter wheat which had been grown with oilseed rape in the previous year. In 1996 and 1997, on 18 March and 20 February, respectively, a total of 60 ground photoeclectors (emergence cages), each covering an area of 0.25 m², were established on sub-plots of ca. 0.3 ha and emptied every third day until 18 June.

Yellow water traps (33 x 25 x 7 cm) and Malaise traps were used to monitor the period of flight activity of adult parasitoids at both the emergence sites and the oilseed rape crops. Eight yellow water traps were ½-filled with tap water and a few drops of detergent. They were positioned on each side of the plot, about 20 m from the crop border. To obtain information on the vertical distribution of parasitoids within the rape crop, four of these water traps were positioned at ground level and four traps were continuously adjusted to the top of the crop canopy. One Malaise trap was placed in the centre of each plot. Trapping started in mid February (mid March in 1996) and was continued until the end of June. The period that parasitoids were present on either crop was assessed by recording the date of the first and final capture of adults in any type of trap. All tersilochine parasitoids were identified to species level according to Horstmann (1971, 1981). As males of *T. microgaster* and *T. obscurator* can not be separated exactly by morphological characters, the evaluation of these species was restricted to females.

Results and discussion

In all three years of study, the timing of emergence and migration of tersilochine parasitoids showed a species-specific sequence. Females of *T. microgaster* were recorded during the early bud stage of oilseed rape in March (Figure 1). Only in 1996, when extended frost temperatures occurred until end of March, was the emergence of *T. microgaster* delayed until early April. Females of *T. obscurator, T. fulvipes* and *P. interstitialis* emerged in all years in early to mid-April and colonized the rape crops from mid-April onwards (Figures 2 – 4). Females of *T. heterocerus* did not occur before the end of April or early May, i.e. usually not until after the beginning of flowering (Figure 5). These results are consistent with previously reported observations on the phenologies of *T. microgaster* and *T. obscurator* in Czechoslovakia (Sédivy, 1983), Germany (Klingenberg & Ulber, 1994) and the UK (Barari *et al*., 2005, Williams *et al*., 2006) and with reports on the seasonal occurrence of *P. interstitialis* and *T. heterocerus* in Germany (Klingenberg & Ulber, 1994), Southern Sweden (Nilsson, 2003; Jönsson *et al*., 2004) and the UK (Ferguson *et al*., 2003).

On some occasions, the abundance of parasitoids and the trapping efficiency may have been too low to obtain reliable estimates of the phenologies within the crops and the initiation of dispersal flights. The number of adult *T. microgaster, T. fulvipes, T. obscurator* and *P. interstitialis* caught in Malaise traps was substantially higher than the number caught in water

traps. The vertical lateral openings of the Malaise trap are much wider than the surface of water traps; however, as the height of these openings measures only 95 cm, catches of parasitoids foraging higher up on top of the crop canopy, like *T. heterocerus*, may have been underestimated by Malaise traps. The vertical position of yellow water traps in the crop had an influence on trapping efficiency. Yellow water traps placed at ground level yielded higher numbers of *T. obscurator* and *T. microgaster* than water traps placed at the top of crop canopy. In contrast, more *T. heterocerus* were captured in traps at the top of the crop canopy than on the ground. Adults of *T. fulvipes* and *P. interstitialis* were found in similar numbers at ground level and on the top of the crop canopy. Yellow water traps attract parasitoids by their colour, which becomes less effective at flowering.

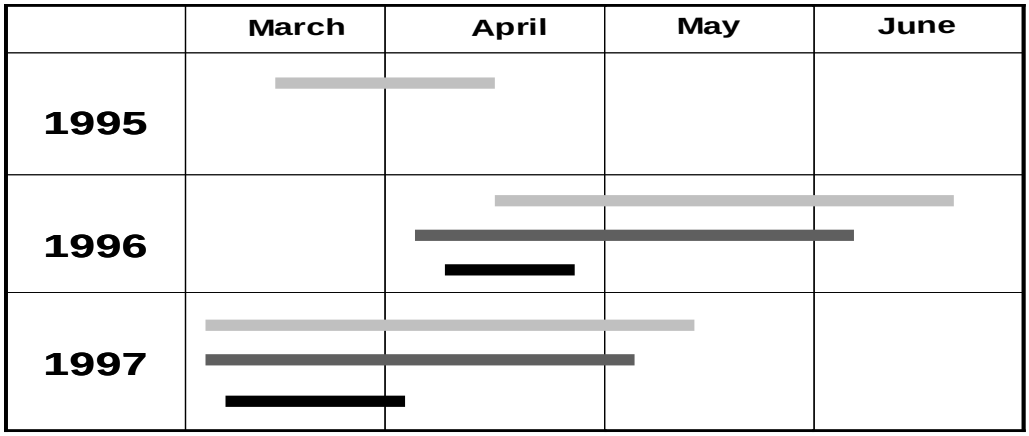


Figure 1. Phenologies of *Tersilochus microgaster* at overwintering sites and on crops of oilseed rape in 1995 – 1997. Period of emergence ▬ ; activity at overwintering site ▬ ; activity on oilseed rape ▬ .

Our results add new information about the effects of climatic factors on parasitoid emergence and flight activity and on the period of the occurrence of parasitoids at emergence sites as well as on crops of oilseed rape. At overwintering sites, the period of emergence and activity varied widely between individual species and years, covering up to 10 weeks. The temporal pattern of emergence and dispersal of parasitoids to new habitats is influenced mainly by their physiological condition and by climatic conditions (Jourdheuil, 1960; Johnen & Ulber, 2004). It is particularly delayed and prolonged when the soil temperature is particularly low in spring. Furthermore, because of the variation in soil depths that parasitoid cocoons were buried by soil cultivation in autumn, the emergence of adults in spring is extended over a long period. Results by Klingenberg & Ulber (1994) indicated that emergence of *T. microgaster* and *T. obscurator* is initiated by maximum daily temperatures of 15 ^{o}C and 17 ^{o}C, respectively. Nilsson (2003) reported on marked differences between times of emergence of adult *P. interstitialis* and *T. heterocerus* when the larvae had developed in pollen beetle larvae either on spring rape or on winter rape.

At the overwintering sites the last specimens of all parasitoids were captured up to 30 days following the end of emergence. The extended period of parasitoid activity at overwintering sites, which in some instances was as long as on oilseed rape, indicates a long lasting period of dispersal between crops. This behaviour is likely to decrease the risk of insufficient coincidance with suitable hosts in crops of oilseed rape and might ensure the complete exploitation of food resources. Emigration flights of *T. obscurator* and *P. interstitialis* from plots of oilseed rape following colonization in April and May have been reported by Williams *et al.* (2006).

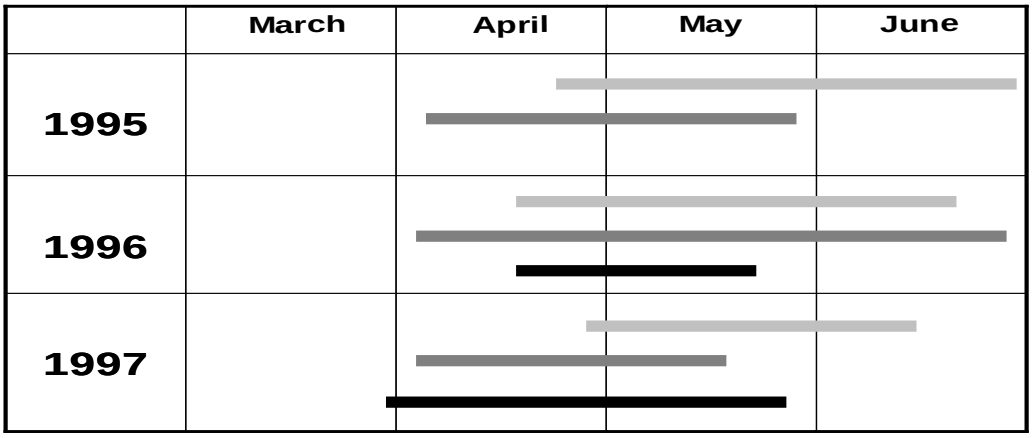


Figure 2. Phenologies of *Tersilochus obscurator* at overwintering sites and on crops of oilseed rape in 1995 – 1997 (legends see Figure 1).

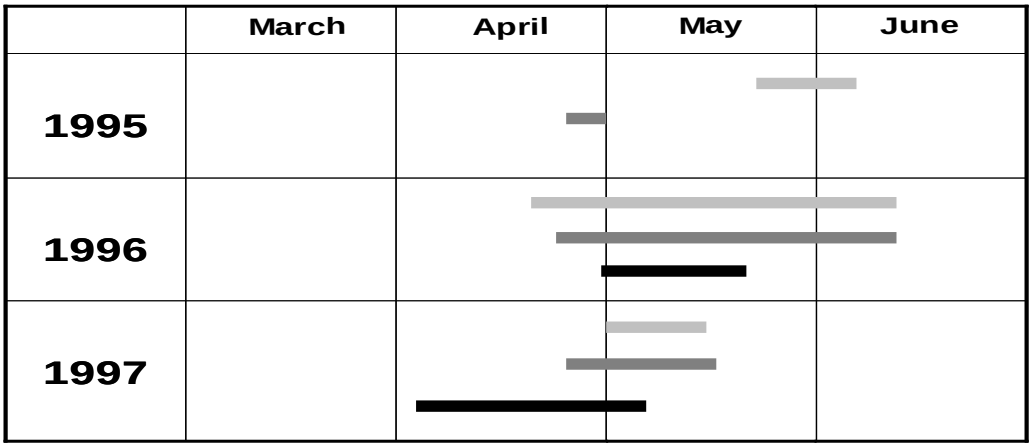


Figure 3. Phenologies of *Tersilochus fulvipes* at overwintering sites and on crops of oilseed rape in 1995 – 1997 (legends see Figure 1).

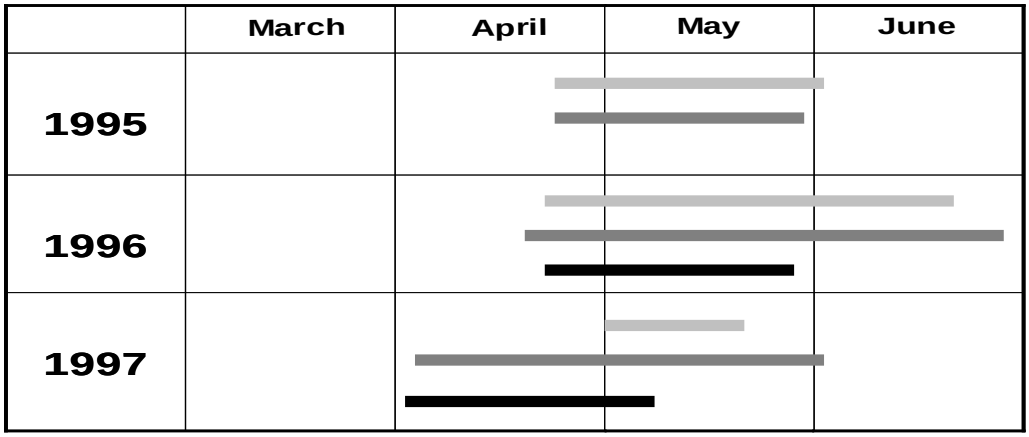


Figure 4. Phenologies of *Phradis interstitialis* at overwintering sites and on crops of oilseed rape in 1995 – 1997 (legends see Figure 1).

The period from the first records of specimens at the emergence site to the first records on nearby crops of oilseed rape varied between species and years, ranging between 0 – 28 days. Commonly, the parasitoids appeared in the oilseed rape crop at the same time or only a few days following the first emergence. In our study, this coincidence may have been supported by relatively small distances (200 – 500 m) between the emergence sites and the oilseed rape plots. Landscape factors can affect the time of arrival of parasitoids in the rape crop (Thies *et al*., 1997).

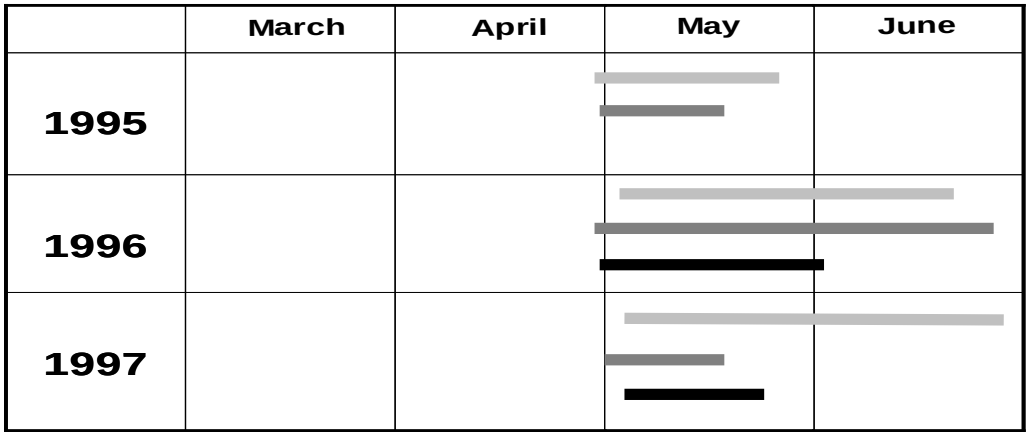


Figure 5. Phenologies of *Tersilochus heterocerus* at overwintering sites and on crops of oilseed rape in 1995 – 1997 (legends see Figure 1).

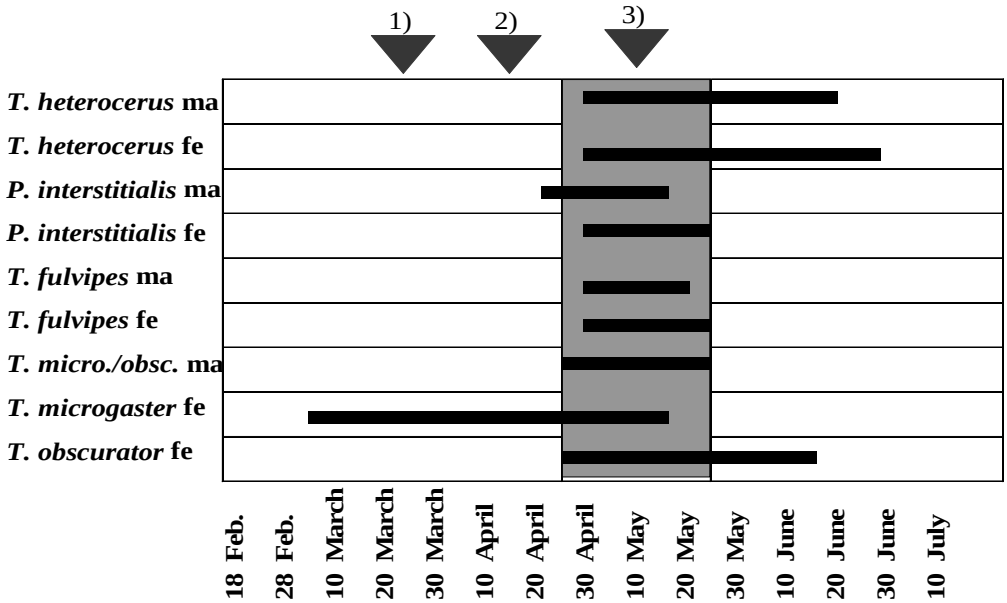


Figure 6**:** Period of activity of tersilochine parasitoids (black bars) in relation to flowering of oilseed rape and insecticide application in 1997. The grey column indicates the period of flowering, the triangles (▼) indicate potential dates of insecticide applications; (ma = males, fe = females)

The period of main activity of tersilochine parasitoids (except *T. microgaster*) on crops of oilseed rape persisted from the late bud stage to the end of flowering. Peak activity of all

tersilochine parasitoids was mainly confined to the period of full flowering of the crop (Figure 6). Consequently, insecticide sprays applied just before or during flowering (Figure 6; insecticide applications 2 and 3) potentially have the most adverse effects on parasitoid populations (Johnen & Ulber, 2004). The avoidance of insecticide application during the flowering period could reduce negative effects on natural control of pest populations in Integrated Pest Management systems. The information on the phenologies of parasitoids at overwintering sites and on crops of oilseed rape provides valuable insight that is essential for establishing predictive models on the periods of occurrence in the crop and in defining spray windows.

Acknowledgements

This study was funded by the EU project MASTER (QLK5-CT-2001-01447) and by the German Research Foundation (DFG) within the Research Training Group 'Agriculture and the Environment'.

References

Barari, H., Cook, S.M., Clark, S.J. & Williams, I.H. 2005: Effect of turnip rape (*Brassica rapa*) trap crop on stem-mining pests and their parasitoids in winter oilseed rape (*Brassica napus*). – BioControl 50: 69-86.

Ferguson, A.W., Campbell, J.M., Warner, D.J., Watts, N.P., Schmidt, J.E.U. & Williams, I.H. 2003: Spatio-temporal distributions of *Meligethes aeneus* and its parasitoids in an oilseed rape crop and their significance for crop protection. – Proceedings of the 11th International Rapeseed Congress, 6-10 July 2003, Copenhagen, Denmark: 1057-1059.

Horstmann, K. 1971: Revision der europäischen Tersilochinen (Hym.: Ichneumonidae). Teil I. – Veröffentlichungen der Zoologischen Staatssammlung München 15: 47-138.

Horstmann, K. 1981: Revision der europäischen Tersilochinae II (Hymenoptera, Ichneumonidae). – Spixiana, Supplementum 4: 1-76.

Jönsson, M., Nilsson, C. & Anderson, P. 2004: Occurrence of pollen beetle parasitoids in the south of Sweden. – IOBC/wprs Bulletin 27 (10): 239-242.

Johnen, A. & Ulber, B. 2004: Perspektiven der Nützlingsschonung im Winterraps durch die Entwicklung von phänologischen Modellen. – Mitteilungen der Biologischen Bundesanstalt für Land- und Forstwirtschaft 396: 278.

Jourdheuil, P. 1960: Influence de quelques facteurs écologiques sur les fluctuations de population d'une biocénose parasitaire: etude relative a quelques hymenopteres (Ophioninae, Diospilinae, Euphorinae) parasites de divers coleopteres infeodes aux cruciferes. – Annales des Épiphyties 11: 445-658.

Kidd, N.A.C. & Jervis, M.A. 1996: Population dynamics. – In: Jervis, M.A. & Kidd, N.A.C. (eds.). Insect natural enemies – practical approaches to their study and evaluation, Chapman & Hall, London: 293-374.

Klingenberg, A. & Ulber, B. 1994: Untersuchungen zum Auftreten der Tersilochinae (Hym., Ichneumonidae) als Larvalparasitoide einiger Rapsschädlinge im Raum Göttingen 1990 und 1991 und zu deren Schlupfabundanz nach unterschiedlicher Bodenbearbeitung. – Journal of Applied Entomology 117: 287-299.

Nilsson, C. 2003: Parasitoids of pollen beetles. – In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Science, Oxford, UK: 73-86.

Nitzsche, O. 1998: Auftreten und Effizienz von Parasitoiden als natürliche Gegenspieler von Schadinsekten im Winterraps unter besonderer Berücksichtigung unterschiedlicher Bodenbearbeitungsmaßnahmen nach Winterraps. – PhD Thesis, University of Göttingen, Göttingen, Germany.

Sédivy, J. 1983: Tersilochinae as parasitoids of insect pests of winter rape (Hymenoptera: Ichneumonidae). – Contributions of the American Entomological Institute 20: 266-276.

Thies, C., Denys, C., Tscharntke, T. & Ulber, B. 1997: Der Einfluß von Saumbiotopen und Ackerbrachen auf Schädlings-Nützlings-Interaktionen im Raps (*Brassica napus* spp. *oleifera*). – Verhandlungen der Gesellschaft für Ökologie 27: 393-398.

Ulber, B. 2003: Parasitoids of ceutorhynchid stem weevils. – In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Science, Oxford: 87-95.

Ulber, B. & Williams, I.H. 2003: Parasitoids of flea beetles. – In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Science, Oxford: 126-138.

Williams, I.H., Büchs, W., Hokkanen, H., Klukowski, Z., Luik, A,. Menzler-Hokkanen, I., Nilsson, C. & Ulber, B. 2004: The EU project MASTER (MAnagement STrategies for European Rape pests): a review of progress. – IOBC/wprs Bulletin 27 (10): 3-16.

Williams, I.H., Barari, H. & Frearson, D. 2006: Upwind anemotaxis by the parasitoid *Tersilochus obscurator* (Hym., Ichneumonidae) on its migration flights to winter oilseed rape. – IOBC/wprs Bulletin 29(7): 181-185.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 181-185

Upwind anemotaxis by the parasitoid *Tersilochus obscurator* (Hym., Ichneumonidae) on its migration flights to winter oilseed rape

Ingrid H. Williams, Hassan Barari, Dave Frearson
Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

Abstract: The direction of migration flights of parasitoids to a crop can affect their subsequent spatial distributions on the crop and hence their effectiveness in conservation biocontrol strategies. The effect of wind direction on the migration flights of natural populations of *Tersilochus obscurator* Aubert (Hym., Ichneumonidae), a parasitoid of the stem-mining pest of oilseed rape, *Ceutorhynchus pallidactylus* (Marsham) (Col., Curculionidae), to a crop of oilseed rape (*Brassica napus* L.) was investigated. Double-sided Malaise traps encircled a crop (20 m diameter) of winter rape, one at each of eight compass points and at 5 m from the crop periphery. Insects were trapped during eight alternate weeks from mid-March to late June 2003. Meteorological data were recorded close to the plot. *Tersilochus obscurator* were caught in the traps from mid April until mid June with a peak in late May. Most (88%) were caught in the external halves of the traps indicating a net movement towards the crop. On all five days analysed, there was a negative correlation between insect catch in the external halves of the traps and air flow through the traps, this was highly significant on three days. This study provides the first field evidence that *T. obscurator* utilizes upwind anemotaxis to locate its host habitat. Understanding the effect of wind direction on parasitoid migration flights to winter rape holds potential for forecasting their arrival and spatial distributions on the crop as well as for manipulating and enhancing their populations for IPM strategies incorporating biocontrol.

Key words: *Brassica napus*, immigration flights, wind direction

Introduction

A better understanding of how parasitoids migrate to winter oilseed rape (*Brassica napus* L.) is required for the development of the integrated pest management strategies incorporating conservation biocontrol that are currently being developed for the crop (Williams, 2004; Williams *et al*., 2004, 2005). The directions of migratory flights influence not only the ability of parasitoids to find the crop but also their subsequent spatial distributions on it (Ferguson *et al*., 2005) and hence their potential for biocontrol of pests.

The larval endoparasitoid *Tersilochus obscurator* Aubert (Hym., Ichneumonidae) is univoltine. It emerges in the spring from overwintering sites in the soil below a previous oilseed rape crop and must migrate to the current year's oilseed rape to find its host, the larvae of the stem-mining pest, *Ceutorhynchus pallidactylus* (Marsham) (Col., Curculionidae), the cabbage stem weevil (Ulber, 2003; Barari *et al*., 2004).

The objective of this study was to determine whether wind direction affects the direction of migratory flights of *T. obscurator* toward a crop of winter oilseed rape.

Materials and methods

A circular plot (20 m diameter) of winter oilseed rape (cv. Lutin) was sown in August 2002 in an open arable landscape on Rothamsted Farm, Hertfordshire. The plot was encircled by eight double-sided Malaise traps (Marris House Nets, Bournemouth, UK), positioned 5 m

from the circumference of the plot and along radii extending from the plot centre in different compass directions (N, NE, E, SE, S, SW, W and NW). Each trap was divided into two halves, external and internal (with respect to the plot), by a central longitudinal mesh partition and sampled insects moving towards and away from the plot, respectively. A meteorological station was set up close to the experimental plot to measure wind direction and wind speed.

Insects collected in the traps were removed daily, from Tuesday to Friday, during eight alternate weeks from 18 March to 27 June 2003. The numbers of *T. obscurator* caught per trap per day were determined. The air flow passing through the open face of each trap was calculated as described in Barari (2005). The correlation between daily trap parasitoid catch and daily trap air flow was tested using Spearman's Rank Correlation. The eight traps were ranked in order of decreasing trap air flow per day and again in order of decreasing numbers of parasitoids caught per day and the ranks compared.

Results and discussion

Upwind anemotaxis, in response to odour cues released from plants on which hosts are feeding, is thought to be an important mechanism by which hymenopterous parasitoids locate their hosts (Vet *et al*., 1995; Vinson, 1998). Oilseed rape plant volatiles, such as the isothiocyanates, have been shown to attract some of the parasitoids of oilseed rape pests when used in baited field traps (Murchie *et al*., 1997) or in olfactometers (Jönsson, 2005), but this is the first direct evidence from the field that a parasitoid of an oilseed rape pest uses upwind anemotaxis to locate its host habitat.

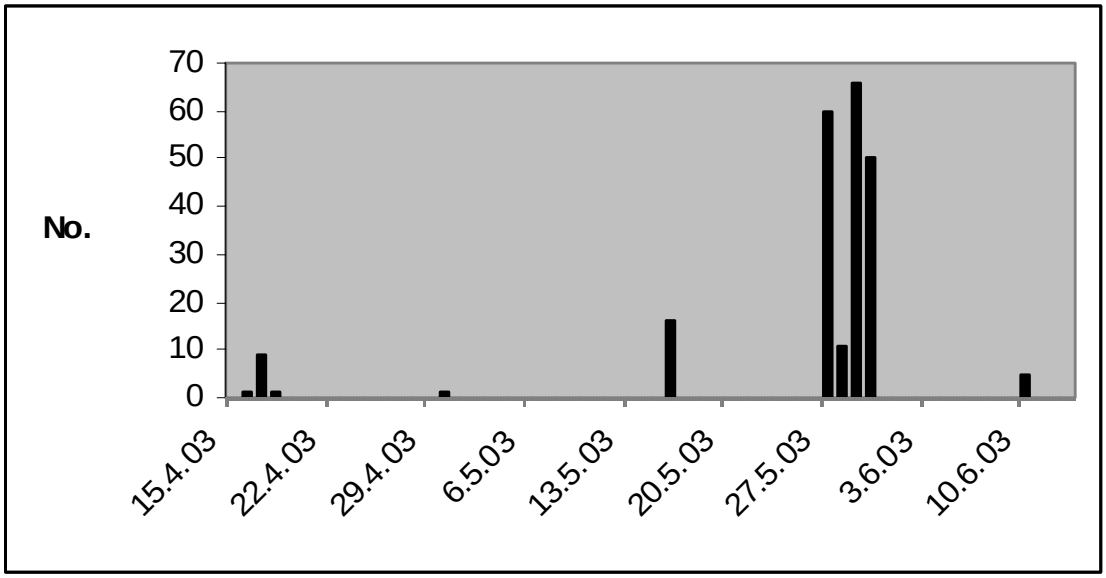


Figure 1. Total numbers of *T. obscurator* caught in external halves of Malaise traps per sampling day.

A total of 249 *T. obscurator* were caught in the Malaise traps from mid April until mid June. Most (88%) of these were in the external halves of the traps with peak numbers at the end of May, indicating a net immigration to the oilseed rape plot during this period (Figure 1). This time of occurrence and immigration agrees with another UK study (Ferguson *et al*., 2005) in which new generation *T. obscurator* adults were caught in emergence traps from mid

April until mid May, following their winter pupation in the soil, and prior to their migration to a new oilseed rape crop.

Table 1. Spearman's Rank Correlation Coefficients (r_s) between air flow through, and the numbers of *T. obscurator* caught in the external halves of 8 Malaise traps on 5 days in 2003.

Date	Total no. of parasitoids	Trap with maximum air flow	Traps with maximum parasitoid catch	r_s	P	Level of sig.
16 May	16	S	NW/NE/SW	-0.12	0.775	NS
27 May	60	SW	NE	-0.86	0.007	**
28 May	11	SW	NW/NE	-0.60	0.116	NS
29 May	66	SE	NW	-0.91	0.002	**
30 May	50	SE	NW	-0.87	0.005	**

Sufficient numbers of *T. obscurator* were caught in the external halves of the Malaise traps for Spearman's Rank Correlation analyses, comparing the ranking of air flow strength through each trap and the numbers of insects caught, on five days during May (Table 1). On all 5 days, air flow was greatest into S, SW or SE traps, indicating wind predominantly from a southerly direction. By contrast, parasitoid catch was greatest into external halves of NE and NW traps suggesting that the direction of flight of the parasitoids towards the crop was against the wind (Table 1). The numbers caught in, and the air flow into each trap on 29 May are shown in Figure 2.

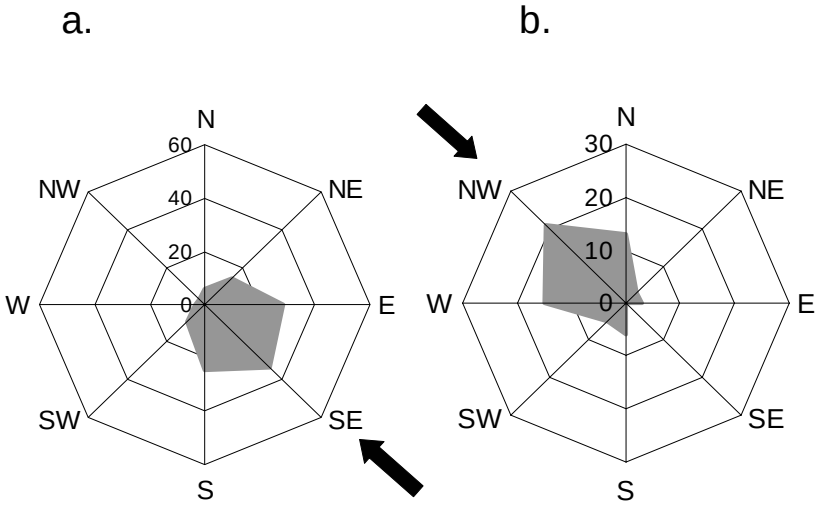


Figure 2. The air flow (1000 m^3) into the external half of each trap (a) and the numbers of *T. obscurator* caught per trap (b). Arrows show main directions of wind (a) and flight (b).

On all five days, there was a negative correlation between trap parasitoid catch and trap air flow; this was very weak on 16 May, modest on 28 May, strong on 27 May and 30 May and very strong on 29 May. The coefficient was not significant on two days, 16 and 28 May, but was highly significant on 27, 29 and 30 May. This confirmed a strong negative relationship between the direction of flight of *T. obscurator* and the direction of the wind.

Understanding the effect of wind direction on parasitoid migration flights to winter rape holds potential for improving the precision of forecasting their arrival and spatial distributions on the crop as well as for manipulating and enhancing their populations for IPM strategies that aim to incorporate conservation biocontrol (Williams, 2004; Williams *et al*., 2004, 2005).

Acknowledgements

We thank Alastair McCartney for advice on calculation of air flow through traps and Suzanne Clark for advice on statistical analysis of data. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK. This study was funded by the EU Framework 5 Quality of Life and Management of Living Resources project MASTER (QLK5-CT-2001-01447) 'Integrated pest management strategies incorporating bio-control for European oilseed rape pests' and by a scholarship from the Iranian Government to Hassan Barari.

References

Barari, H. 2005: Ecology of the coleopteran stem-mining pests and their parasitoids in winter oilseed rape: implications for integrated pest management. – Unpublished PhD thesis, University of London.

Barari, H., Cook, S.M. & Williams, I.H. 2004: Rearing and identification of the larval parasitoids of *Psylliodes chrysocephala* and *Ceutorhynchus pallidactylus* from field-collected specimens. – Bulletin IOBC/wprs 27(10): 263-272.

Ferguson, A.W., Barari, H., Warner, D.H., Campbell, J.M., Smith, E. T. & Williams, I.H. 2005: Phenologies and spatial distributions of the stem miners, *Psylliodes chrysocephala* (L.) and *Ceutorhynchus pallidactylus* (Marsham), and their parasitoids in a crop of winter oilseed rape (*Brassica napus* L.). – Ent. Exp. et Appl. (submitted)

Jönsson, M. 2005: Responses to oilseed rape and cotton volatiles in insect herbivores and parasitoids. – PhD thesis No.2005:14 Swedish University of Agricultural Sciences, Alnarp, Sweden.

Murchie, A.K., Smart, L.E., & Williams, I.H. 1997: Responses of *Dasineura brassicae* and its parasitoids *Platygaster subuliformis* and *Omphale clypealis* to field traps baited with organic isothiocyanates. – J. Chem. Ecol. 23: 917-926.

Ulber, B. 2003: Parasitoids of Ceutorhynchid Stem Weevils. – In: Alford, D. (ed.): Biocontrol of Oilseed Rape Pests Blackwell Publishing, Oxford: 9-42.

Vet, L.E.M., Lewis,W.J. & Cardé,R.T. 1995: Parasitoids foraging and learning. – In: R.T. Cardé & W.J. Bell (ed.). Chemical Ecology of Insects. 2. New York, Chapman and Hall: 65-104.

Vinson, S.B. 1998: The general host selection behaviour of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. – Biological Control 11: 79-96.

Williams, I.H. 2004: Advances in insect pest management of oilseed rape in Europe. – In: A.R. Horowitz & I. Ishaaya (eds.). Insect Pest Management. Berlin, Springer-Verlag: 181-208.

Williams, I.H., Büchs, W., Hokkanen, H., Klukowski, Z., Luik, A., Menzler-Hokkanen I., Nilsson, C. & Ulber, B. 2004: The EU project MASTER (MAnagement STrategies for European Rape pests): a review of progress. – Bulletin IOBC/wprs 27(10): 3-16.

Williams, I.H, Büchs, W., Hokkanen, H., Johnen, A., Klukowski, Z., Luik, A., Menzler-Hokkanen, I., Nilsson, C. & Ulber, B. 2005: MASTER – Integrating biological control within IPM for winter oilseed rape across Europe. – The BCPC Conference, Glasgow, 31 October-2 November 2005.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 187-191

Phradis morionellus on *Meligethes aeneus*: Long-term patterns of parasitism and impact on pollen beetle populations in Finland

Heikki M.T. Hokkanen
Department of Applied Biology, Helsinki University, Box 27, FIN-00014 Helsinki, Finland
heikki.hokkanen@helsinki.fi

Abstract: Data on annual percent parasitism of *Meligethes aeneus* (Nitidulidae) larvae by *Phradis morionellus* (Ichneumoniidae) were collected in Finland for 11 years (1985-1995) from 35-70 different locations (13 regions) covering the total area of rapeseed cropping in Finland. Information on the level of pest attack at the national level (severity and frequency of attack) was obtained from the annual pest survey data, carried out by the Agricultural Research Centre of Finland until 1992. Total proportion of pollen beetles removed from the emerging new generation adult population by parasitism (%-parasitism at each region weighted by the area of rapeseed grown in that area) varied between 49.5% in 1987 to 7.5% in the following year (1988); usually the proportion was around 30%. Pollen beetle attack decreased from severe in the early 1980s to moderate in 1985-88 with rising levels of parasitism by *P. morionellus*. After the 1988 crash in *Phradis* populations, pollen beetle attack jumped again to severe until the early 1990s, after which some balance appears to have been reached. Insecticide sprays to control the all-time high populations of the cereal aphid *Rhopalosiphum padi* in 1988 are a likely explanation for the parasitoid crash in this year. A dynamic simulation model was constructed to describe the rapeseed plant, pollen beetle, and parasitoid interaction. This suggested that pollen beetle populations indeed can be held at a very low level by *Phradis*, if the interaction is not disrupted with pesticide applications, or if the parasitoid is enhanced by some other simple means. The parasitoid dynamics appear to follow a surprising pattern, being in synchrony over several large regions, but varying widely between these larger regions. Edaphic and climatic factors may explain such regional patterns. Also, several micro-level factors were identified as influencing the level of parasitism at an individual-field level. These include distance to forest edge, soil type, size of rapeseed field, and possibly the abundance of umbelliferous plants at the field edge.

Key words: Annual cropping systems, climatic factors, edaphic factors, insect parasitoids, integrated control, pest management, pollen beetle, population dynamics, soil type, turnip rapeseed

Introduction

Detailed information on pest-natural enemy dynamics over longer periods of time from annual cropping systems are very scarce, particularly regarding the factors determining the level of control exercised by the natural enemies in such unstable habitats. Theoretical and practical studies on host-parasitoid interactions usually focus on more stable ecosystems such as forests or orchards (e.g., Hassell & Wilson, 1997), and virtually no such studies take into account the potential role of abiotic factors as determinants of the actual field levels of natural enemy efficacy.

In Finland, the natural control factors and management of the pollen beetle *Meligethes aeneus* F. have been studied by my group since 1983 (see, e.g. Hokkanen *et al*., 1986, 1988; Hokkanen, 1993, 2000; Hokkanen & Lipa, 1995). The aim of this study was to detect patterns in long-term percent parasitism of the pollen beetle by *Phradis morionellus* (Holm.) (Hym., Ichneumonidae) over the whole rapeseed area in Finland, to relate any such patterns to abiotic

and biotic variables such as geographic regions or biotic zones, and to identify local factors, which might explain variations in the observed levels of parasitism at the local level.

Materials and methods

Data on annual percent parasitism of pollen beetle larvae by *P. morionellus* were collected in Finland for 11 years (1985-1995) from 35-70 different locations (13 regions) covering the total area of rapeseed cropping in Finland. At each location, larval samples were collected from five different spots. The larvae were placed in a plastic cup with a rapeseed inflorescence, stored in a cool box for transport, and were transferred to the laboratory for processing. From each location, 30-100 larvae were dissected under a microscope, depending on the level of parasitism, until a 95% reliability of the parasitism level was reached. In some years, additional information was collected at each sampling site on: distance to forest edge, soil type, size of rapeseed field, and the abundance of umbelliferous plants at the nearest field edge. Information on the level of pest attack at the national level (severity and frequency of attack) was obtained from the annual pest survey data, carried out by the Agricultural Research Centre of Finland until 1992.

Results and discussion

Total proportion of pollen beetles removed (*sensu* van Driesche *et al.*, 1991) from the new generation adult population (%-parasitism at each region weighted by the area of rapeseed grown in that area) varied between the highest level in 1987 at 49.5% to the lowest in the following year (1988) at 7.5%; usually the proportion was around 30% (Fig. 1). Pollen beetle attack decreased from severe in the early 1980s to moderate in 1985-88, with rising levels of parasitism by *P. morionellus*. After the 1988 crash in *Phradis* populations, pollen beetle attack jumped again to severe until the early 1990s, after which some balance appears to have been reached (Fig. 1). Insecticide sprays to control the all-time high populations of the cereal aphid *Rhopalosiphum padi* in 1988 are a likely explanation for the parasitoid crash in 1988.

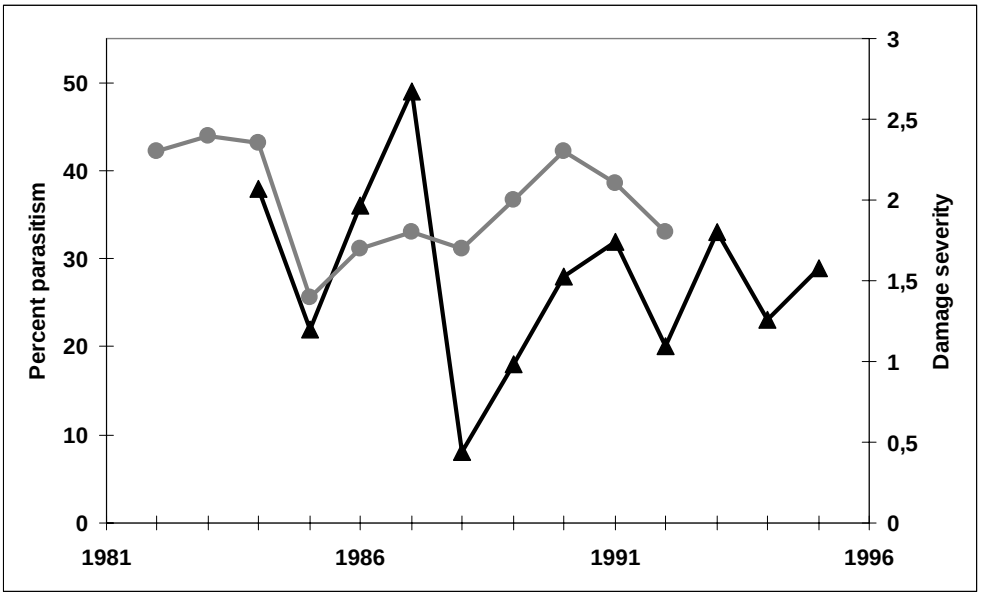


Figure 1. Pollen beetle overall damage severity 1982-1992 (grey line, dots) and percent parasitism 1984-1995 (black line, triangles) in Finland. Damage severity data: Agricultural Research Centre of Finland survey data.

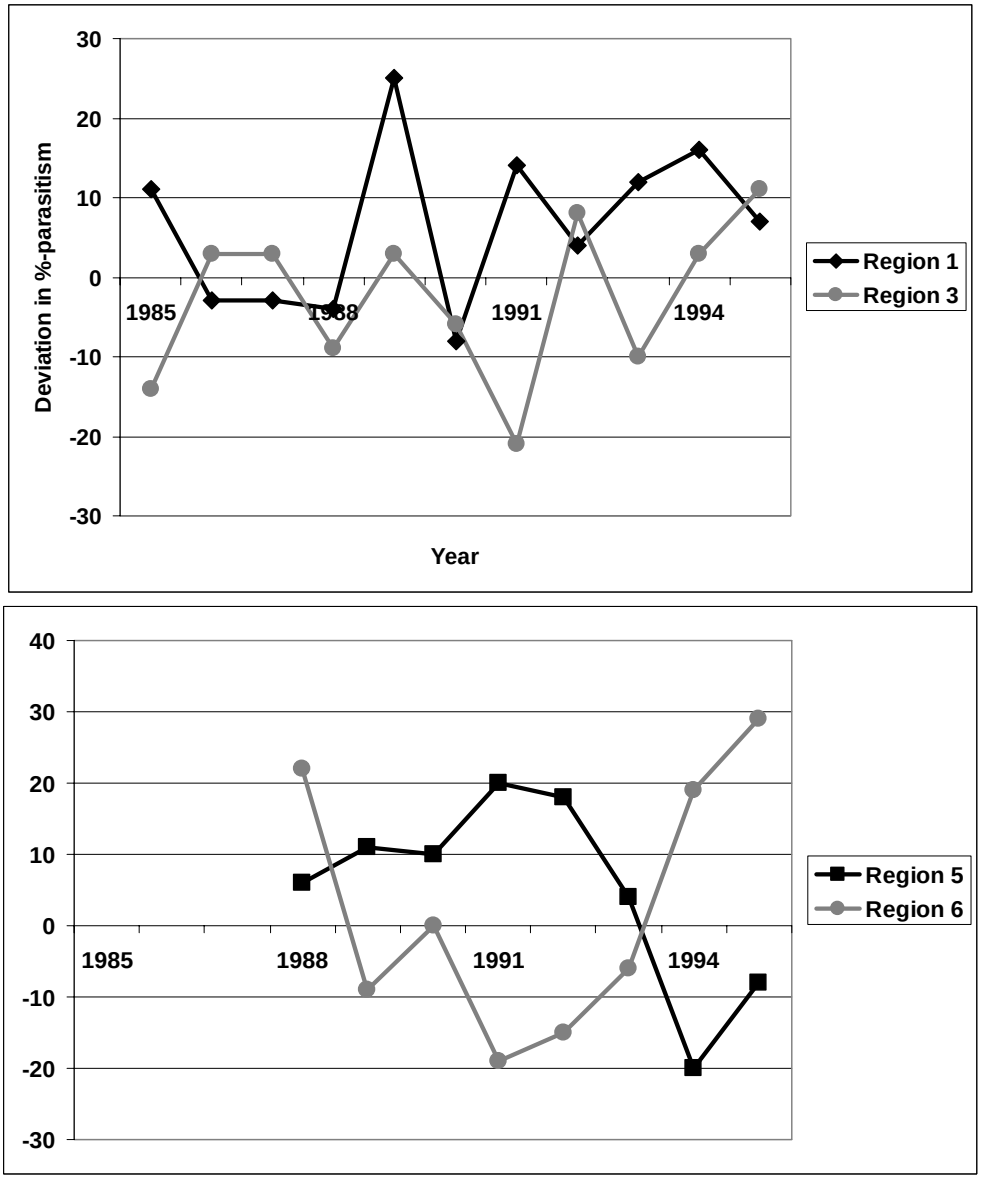


Figure 2. Examples of regional patterns in pollen beetle parasitism: deviations from the annual overall (national) percent parasitism in 1985-1995 for regions 1 and 3, and in 1988-1995 for regions 5 and 6. See Fig. 3 for locations of the regions.

Correlation analysis of the time-series data for the 13 sampled regions revealed that the parasitoid dynamics appear to follow a surprising pattern, being in synchrony over several large regions (Figs. 2-3), but varying widely between these larger regions.

What could influence the host-parasitoid dynamics to such an extent that patterns are synchronized over areas larger than 100 km in diameter? As far as I can see, only edaphic and climatic factors could explain such regional patterns. Indeed, the boundaries of relief pattern types, and those of biotic zones in Southern Finland (Fig. 4) are surprisingly similar to the approximate boundaries shown in Fig. 3 for the parasitism dynamics.

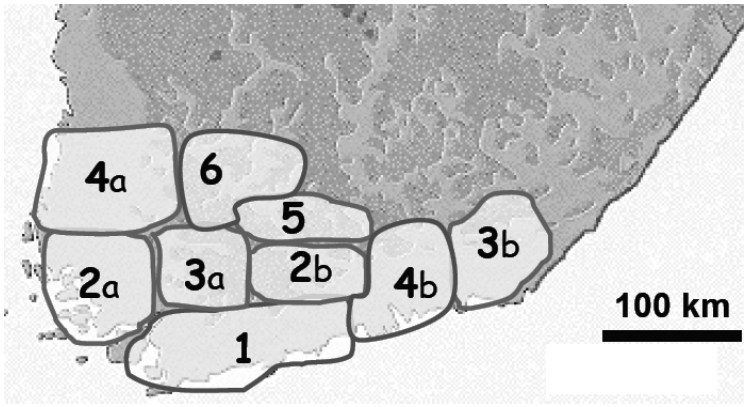


Figure 3. Rapeseed growing areas in Finland, where pollen beetle parasitism by *Phradis morionellus* appear to fluctuate in synchrony within each region. Numbers 1 to 6 represent the six different time-series patterns detected for pollen beetle parasitism; a and b designate geographically separate areas following the same pattern (e.g., 2a and 2b).

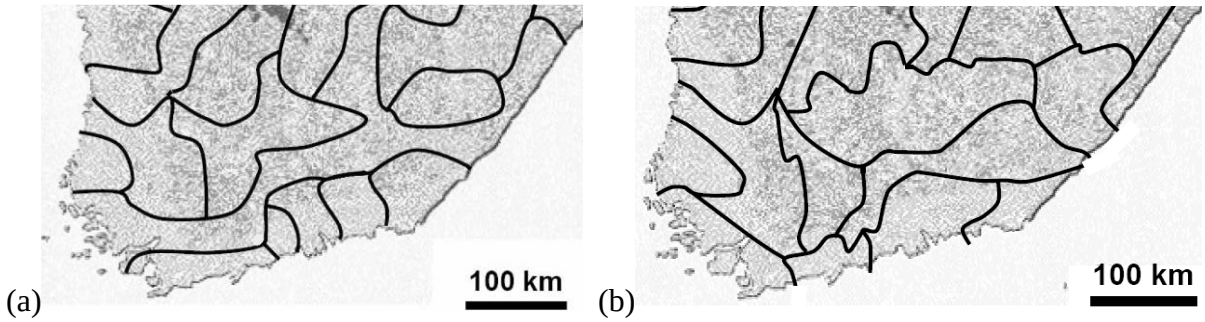


Figure 4. (a) Boundaries of relief pattern types of landscapes in Southern Finland, and (b) boundaries of biotic zones in Southern Finland (after Aario, 1960).

Several micro-level factors were identified additionally as influencing the level of parasitism at an individual field level. These include distance to forest edge, soil type, size of rapeseed field, and the abundance of umbelliferous plants at the field edge (data to be published elsewhere).

Parasitoids appeared to significantly lower the pollen beetle populations as soon as 30-40 % parasitism was reached (Fig. 1). In 1985-88 the drop in pollen beetle damage severity may have been caused by the high levels of parasitism. The parasitoid crash in 1988 appears to have released the pollen beetles from this natural control, which afterwards seemed to catch up only slowly (Fig. 1). To further study this situation, a dynamic simulation model was constructed (Kaukoranta & Hokkanen, unpublished) to describe the rapeseed plant, pollen beetle, and parasitoid interaction. This suggested that pollen beetle populations can indeed be held at a very low level by *Phradis*, if the interaction is not disrupted with pesticide applications, or if the parasitoid population is enhanced by some other simple means. This prediction still awaits for validation in practice, but all available data suggest that in Finland, biocontrol of the pollen beetle is possible using simple conservation biological control methods.

Acknowledgements

Beyond the initial grant from the Academy of Finland (1983-1985), it was not possible to obtain any further funding to carry out this research, despite countless attempts to many funding agencies. I am thus grateful to my employers (Finnish Agricultural Research Centre, 1985-1990, and the University of Helsinki, Department of Applied Zoology, since 1990) for allowing me to continue work on pollen beetle parasitism rates. The annual "pollen beetle rally" throughout Southern Finland could not have been carried out without help from the technical staff: Ms. Silja Mäkelä and Mr. Veli-Matti Väänänen (UH, DAZ). Help from Gun-Britt Husberg, Mona Söderblom, and Helena Granlund at the initial phases of this work has been invaluable. This analysis was completed and the paper written during the EU-project MASTER: MAnagement STrategies for European Rape pests (QLK5-CT-2001-01447).

References

Aario, L. (ed.) 1960: Atlas of Finland. – The Geographical Society of Finland. Otava, Helsinki.

Hassell, M.P. & Wilson, H.B. 1997: The dynamics of spatially distributed host-parasitoid systems. – In: D. Tilman & P. Kareiva (eds.). Spatial Ecology. Princeton University Press, Princeton, N.J.: 75-110.

Hokkanen, H.M.T. 1993: Overwintering survival and spring emergence in *Meligethes aeneus*: effects of body weight, crowding, and soil treatment with *Beauveria bassiana*. – Entomologia Experimentalis et Applicata 67: 241-246.

Hokkanen, H.M.T. 2000: The making of a pest: recruitment of *Meligethes aeneus* onto oilseed brassicas. – Entomologia Experimentalis et Applicata 95: 141-149.

Hokkanen, H., Granlund, H., Husberg, G.-B. & Markkula, M. 1986: Trap crops used successfully to control *Meligethes aeneus* (Col., Nitidulidae), the rape blossom beetle. – Annales Entomologici Fennici 52: 115-120.

Hokkanen, H., Husberg, G.-B. & Söderblom, M. 1988: Natural enemy conservation for the integrated control of the rape blossom beetle *Meligethes aeneus* F. – Annales Agriculturae Fenniae 27: 281-294.

Hokkanen, H.M.T. & Lipa, J.J. 1995: Occurrence and dynamics of *Nosema meligethi* (Microsporida) in populations of *Meligethes aeneus* (Coleoptera, Nitidulidae) in Finland. – Entomologica Fennica 6: 11-18.

van Driesche, R.G., Bellows, T.S., Elkinton, J.S., Gould, J.R. & Ferro, D.N. 1991: The meaning of percentage parasitism revisited: solutions to the problem of accurately estimating total losses from parasitism. – Environ. Entomol. 20: 1-7.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 193-199

Adult activity and larval abundance of stem weevils and their parasitoids at different crop densities of oilseed rape

Nadine Neumann and Bernd Ulber
Department of Crop Sciences, Plant Pathology and Plant Protection Division, Entomology Section, University of Goettingen, Grisebachstrasse 6, D-37077 Goettingen, Germany

Abstract: The effect of oilseed rape plant density on adult activity, larval abundance and larval parasitism of stem weevils, *Ceutorhynchus napi* and *C. pallidactylus*, and their larval parasitoids, *Tersilochus fulvipes* and *T. obscurator* (Hym.: Ichneumonidae), respectively, was studied in a randomised field experiment of two contrasting plant densities (10 plants/m² and 70 plants/m²). Adult activity of weevils and parasitoids was monitored using water trays set up below the crop canopy. The abundance of stem-mining larvae was assessed from plant samples. The level of parasitism was determined by dissection of larvae.

In plots of low plant density, the numbers of adult *C. napi* and *T. fulvipes* trapped in water trays were significantly greater than at the higher plant density. The activity density of *C. pallidactylus* and *T. obscurator* was not affected by plant density. The number of *C. pallidactylus* larvae per plant in plots of low plant density was significantly higher than in plots of high density. The abundance of *C. pallidactylus* larvae per m² was similar at both plant densities, indicating that plant resources needed for oviposition and larval development were sufficiently available, even at low plant density. In contrast to our expectations, the number of *C. napi* larvae per plant did not increase with decreasing plant density. Corresponding to the activity density of adult *T. fulvipes*, percentage parasitism of *C. napi* at low plant density was significantly higher than at high plant density. The level of parasitism of *C. pallidactylus* was not affected by plant density.

Key words: Plant density, oilseed rape, *Ceutorhynchus napi*, *Ceutorhynchus pallidactylus*, *Tersilochus fulvipes*, *Tersilochus obscurator*

Introduction

Sowing rates of winter oilseed rape in Germany in recent years have been reduced substantially, in order to optimize the crop canopy structure and yield of the crop. The plant density aimed for ranges between 30 – 50 plants/m^2, as compared with 80 – 100 plants/m^2 in former years (Sauermann & Gronow, 2000). These changes may have wider consequences for the level of pest infestation and for natural control of pests (Price *et al*., 1980; Cortesero *et al*., 2000). Plant density and plant architecture have been reported to influence the abundance of pest insects of oilseed rape (Finch & Skinner, 1976; Dosdall *et al*., 2003; Nuss, 2004), however, information is limited regarding how they affect parasitism of stem-boring pests.

The rape stem weevil, *Ceutorhynchus napi* Gyllenhal, and the cabbage stem weevil, *Ceutorhynchus pallidactylus* (Marsham) (Col.: Curculionidae) are major pests of winter oilseed rape in Europe. In March and April, they invade the rape crops from their overwintering sites. Females of *C. napi* deposit their eggs singly into developing stems, which result in stunting, twisting and splitting of stem tissue (Alford *et al*., 2003). Females of *C. pallidactylus* lay their egg batches preferably into petioles; the larvae mine the petioles and later invade the stems. The univoltine endoparasitoids *Tersilochus fulvipes* (Gravenhorst) and *Tersilochus obscurator* Aubert (Hym.: Ichneumonidae-Tersilochinae) have been identified as

key larval parasitoids of *C. napi* and *C. pallidactylus*, respectively (Ulber, 2003; Williams *et al*., 2004). They colonize rape crops from their overwintering sites in April and May. Parasitoid females lay their eggs singly through the plant tissue into host larvae while these are mining within stems or petioles. Levels of parasitism by *T. fulvipes* and *T. obscurato*r can be high, however, parasitoid efficiency may be limited by insufficient spatio-temporal coincidence of females with the appearance of accessible host larvae in plants (Ulber, 2003). In this study, oilseed rape plots of two contrasting plant densities were studied to determine if decreasing crop density has a significant effect on populations of stem weevils and their larval parasitoids.

Materials and methods

Field experiment

The effect of plant density and plant architecture on the abundance of *C. napi* and *C. pallidactylus* as well as their parasitoids was studied in a field experiment at Goettingen, northern Germany, in 2004. Two contrasting plant densities (70 plants/m² and 10 plants/m²) of oilseed rape cv. 'Artus' were established in a randomised block design with 6 replicated plots (5 m x 5 m). To achieve a homogeneous crop structure in low-density plots, the plant number of 10 plants/m² was achieved by hand-pulling and hoeing in February.

Activity density

The activity density of adult stem weevils and parasitoids within plots was assessed by water-filled white plastic trays (18 x 13 x 6 cm). Two water trays were positioned on the ground 2 m apart, in the centre of each plot. The traps were emptied every seven days between 26 April and 18 May 2004. Adult weevils and parasitoids were counted and identified.

Larval abundance and parasitism

The abundance of *C. napi* and *C. pallidactylus* larvae was determined from samples of 10 plants collected at random from each plot on 11 May, before larvae started to migrate to the soil for pupation. The larvae were removed from the stems and petioles and stored in 70 % ethanol. The level of parasitism was examined by dissecting the larvae under a microscope. To determine the identity of parasitoid species, sub-samples of larvae were reared to the adult stage.

Results and discussion

Activity density of adult stem weevils and their parasitoids

The number of stem weevils and parasitoids captured during the first week of sampling in all plots was much higher than the number caught in two subsequent weeks (Figure 1 & 2). In the second and third week, decreasing numbers of insects trapped may have been caused by low temperatures and frequent rainfall. In plots of low plant density, the number of adult *C. napi* significantly increased compared to plots of high plant density (Figure 1). Similarly, a significantly higher number of *T. fulvipes* was found at low plant density between 26 April and 3 May. The number of adult *C. pallidactylus* and *T. obscurator* captured in water trays was not affected significantly by plant density (Figure 2). On 3 May, the parasitoid/host ratio of *T. fulvipes* and *C. napi* in plots of 10 plants/m² and 70 plants/m² was 0.03 and 0.07, respectively, whereas the parasitoid/host ratio of *T. obscurator* and *C. pallidactylus* at either plant density was nearly 1.0.

Increased numbers of adult *C. napi* and *T. fulvipes* at 10 plants/m² may indicate that these species have been attracted preferably to areas with sparse vegetation of their host plants.

However, the number of insects caught in water trays is known to depend on both their abundance and locomotor activity. As a result, the trap data obtained from plots of low and high plant density may have been affected by different crop density, microclimate and other factors. Vegetation density can impede the movement rates of insect and the propability that insects encounter the traps (Powell *et al*., 1996). *Tersilochus fulvipes* and *T. obscurato*r have been reported to forage preferably at lower strata of the crop, where they find their hosts in stems and petioles of oilseed rape plants (Nitzsche, 1998). Consequently, the water trays have provided data on the activity rather than on the abundance of adult weevils and parasitoids within plots.

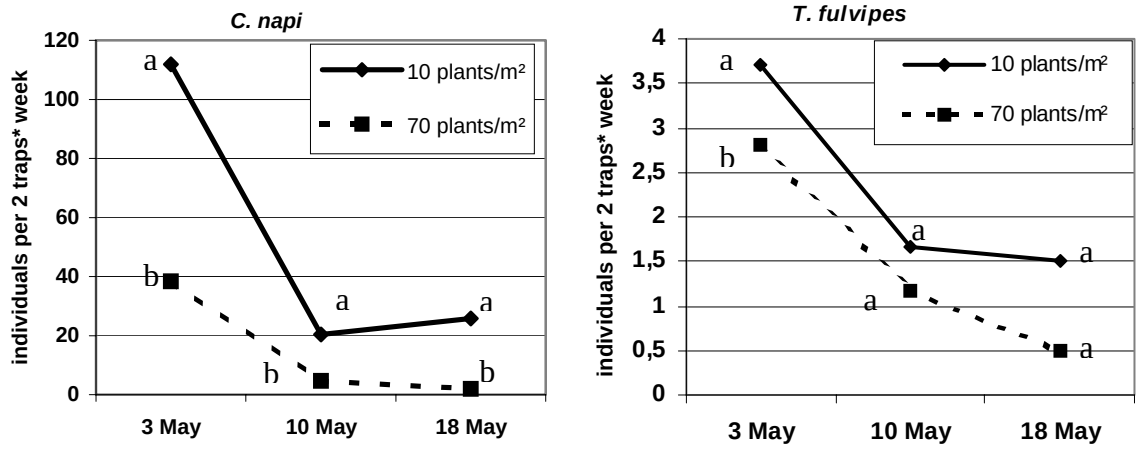


Figure 1. Activity density of adult *C. napi* and *T. fulvipes* in plots of low and high plant density of oilseed rape. Means with the same letters are not significantly different ($P \leq 0.05$)

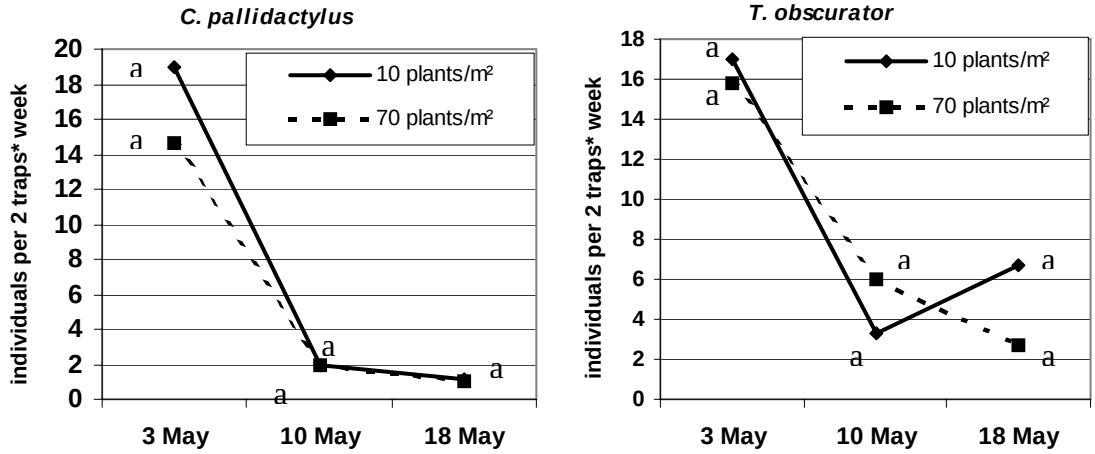


Figure 2. Activity density of adult *C. pallidactylus* and *T. obscurator* in plots of low and high plant density of oilseed rape. Means with the same letters are not significantly different ($P \leq 0.05$)

***Larval abundance of* C. napi *and* C. pallidactylus**

The decrease of plant resources for oviposition and larval development by reducing plant numbers was found to have different effects on the level of infestation by *C. napi* and *C. pallidactylus* (Figure 3 & 4). Contrary to our expactations, the number of *C. napi* larvae per

individual plant in plots of 10 plants/m² was only little higher than in plots of 70 plants/m², in spite of very few plants available for oviposition (Figure 3). The low number of stems and lateral racemes might have increased the intraspecific competition between egg-laying females. Further, an excessive oviposition into individual stems might have increased the mortality of eggs and larvae. On an area level, the abundance of *C. napi* larvae per m² in plots of high plant density was significantly higher than in plots of low plant density (Figure 3).

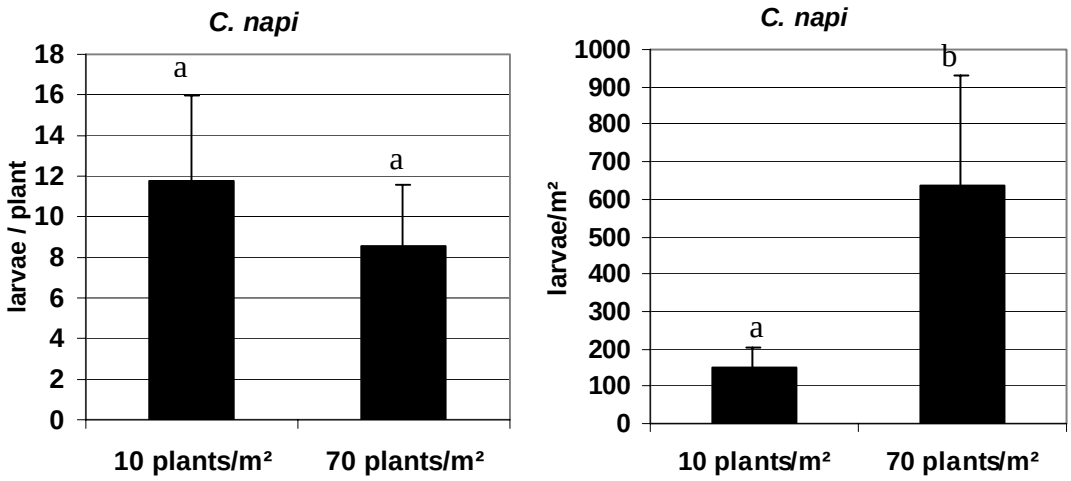


Figure 3. Effect of low and high plant density of oilseed rape on the mean number of *C. napi* larvae per plant (± SD) and the mean number of larvae per m² (± SD). Means with the same letters are not significantly different ($P \leq 0.05$).

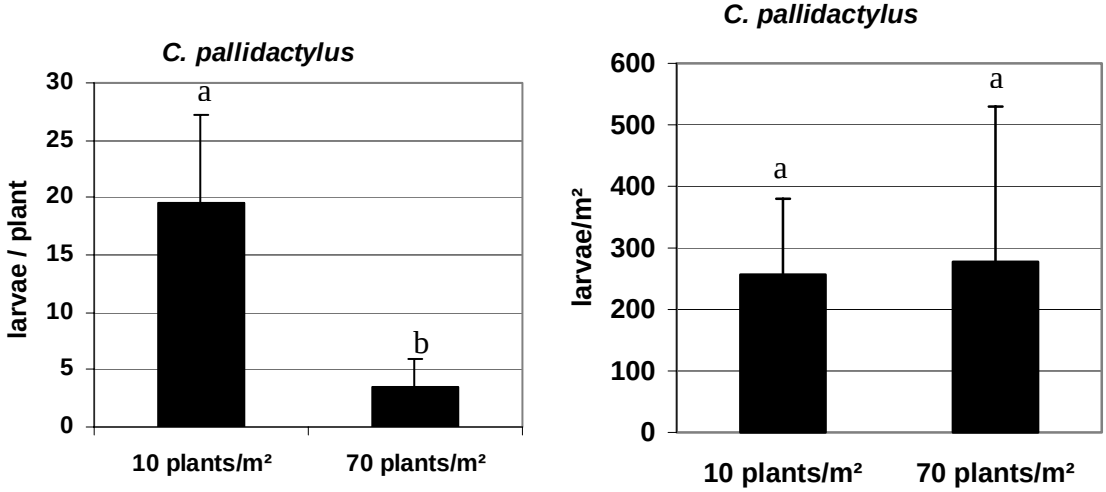


Figure 4. Effect of low and high plant density of oilseed rape on the mean number of *C. pallidactylus* larvae per plant (± SD) and the mean number of larvae per m² (± SD). Means with the same letters are not significantly different ($P \leq 0.05$).

The number of *C. pallidactylus* larvae per plant significantly increased with decreasing plant density ($y = 21.52 - 0.23x$, $P = 0.0005$; $R^2 = 0.57$). In plots of 10 plants/m², the number of larvae per plant was 5.7 times higher than in plots of 70 plants/m² (Figure 4). At 10 plants/m², the number of leaves per plant was significantly increased, and larger petioles supplied sufficient resources for the oviposition of *C. pallidactylus* and for the development

of high numbers of larvae. The abundance of *C. pallidactylus* larvae per m² was on a similar level at eigher plant density (Figure 4), indicating that oviposition and larval performance of this species was not affected even at very low plant density. Ferguson et al. (2003) studied the spatio-temporal distribution of *C. pallidactylus* in oilseed rape and concluded that this species may selectively infest larger plants with larger stems. There was no significant relationship between the activity density of adult stem weevils and subsequent densities of weevil larvae within plants with either species.

***Larval parasitism of* C. napi *and* C. pallidactylus**

Corresponding to the activity density of adult *T. fulvipes*, the percentage parasitism of *C. napi* by *T. fulvipes* increased significantly with decreasing plant density (Table 1). Stems of wider diameter which are more common at low plant density of oilseed rape have been found to provide a structural refuge for stem-boring host larvae against parasitism (Ulber, 2003). Owing to the shortness of the ovipositor, female *T. fulvipes* is unable to penetrate the larvae of *C. napi* when these are feeding in the centre of thick stems. However, at low plant density 34.6 % of larvae developed within lateral racemes, as compared to 2.1 % of larvae at high plant density. There was evidence of increased parasitism of *C. napi* in lateral racemes, probably due to the smaller diameter of racemes which allowed easy access of parasitoid females to host larvae.

Percentage parasitism of *C. pallidactylus* larvae was not affected significantly by plant density (Table 1). At either plant density, host larvae were available and accessible for parasitism for a long period within petioles. Further, the position of larvae within leaves and stems apparently had no influence on percentage parasitism. There was a tendency of increased levels of parasitism of *C. pallidactylus* by *T. obscurator* with increasing host density. On an area-related scale, the number of parasitized *C. pallidactylus* larvae per m² significantly increased with numbers of larvae per m² (Figure 5). There was no evidence for a density-dependent relationships between *C. napi* and *T. fulvipes*. Based on an area scale, the number of parasitized larvae of *C. napi* per m² was not related significantly to the number of host larvae per m² (Figure 5).

Table 1. Parasitism of *C. napi* and *C. pallidactylus* larvae at low and high plant density of oilseed rape. Means followed by the same letters within columns are not statistically significant ($P \leq 0.05$)

Plant density	Mean level of parasitism (%) ( ) = total number of dissected larvae	
	C. napi	*C. pallidactylus*
10 plants/m²	18.3 a (619)	22.9 a (547)
70 plants/m²	5.9 b (306)	18.6 a (145)

There was no significant relationship between the parasitoid/host ratio of adults in water trays and the level of parasitism at low and high plant density. In spite of relatively large numbers of adult *T. obscurator* trapped in water trays and a narrow parasitoid/host ratio the level of parasitism of *C. pallidactylus* by *T. obscurator*, as compared with parasitism of *C. napi*, was not increased. The complex factors influencing trapping efficiency and parasitisation at various plant densities (Cortesero *et al*., 2000) might have interfered with a closer relationship.

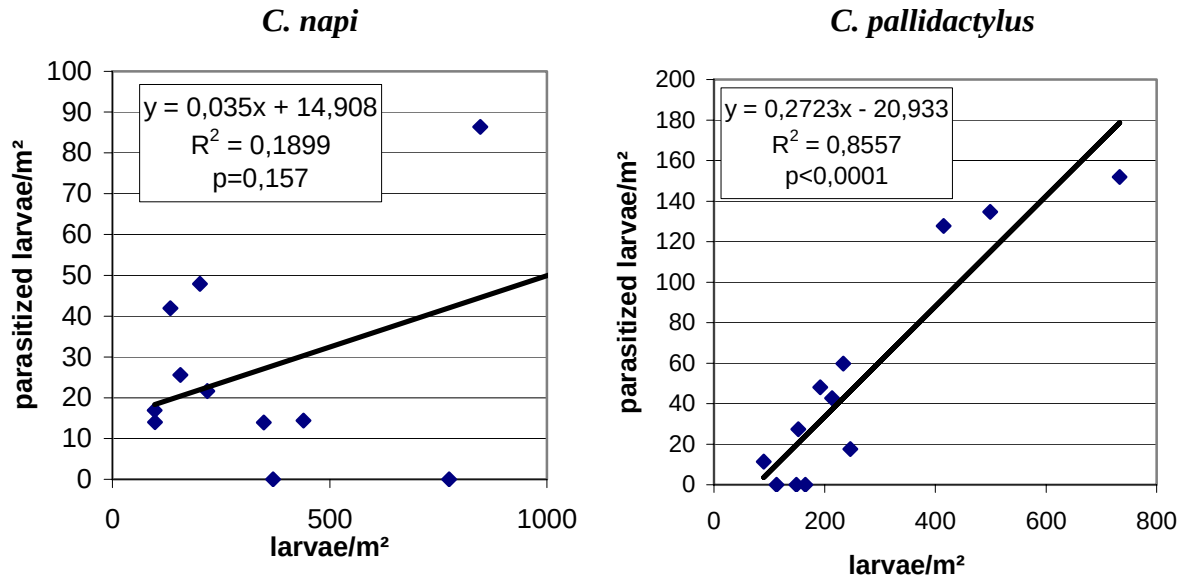


Figure 5. Linear regressions of the relationships between mean numbers of *C. napi* and *C. pallidactylus* larvae per m² and mean numbers of parasitized larve per m² . Larvae were collected from plots of low and high plant density of oilseed rape (n = 12).

Acknowledgements

This study was funded by the EU Project MASTER (QLK5-CT-2001-01447) “Integrated pest management incorporating bio-control for European oilseed rape pests”.

References

Alford, D.V., Nilsson, C. & Ulber, B. 2003: Insect pests of oilseed rape crops. – In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Publishing, Oxford, UK: 9-41.

Dosdall, L.M., Carcamo, H., Kott, l., Kuhlmann, U., Mason, P., Moisey, D. & Olfert, O. 2003: Progress towards integrated management of cabbage seedpot weevil in western Canada. – In: Proc. 11th Int. Rapeseed Congress, Copenhagen 3: 1022-1024.

Cortesero, A.M., Stapel, J.O. & Lewis, W.J. 2000: Understanding and manipulating plant attributes to enhance biological control. – Biological Control 17: 35-49.

Ferguson, A., Klukowski, Z., Walczak, B., Clark, S.J., Mugglestone, M.A., Perry, J.N. & Williams, I.H. 2003: Spatial distribution of pest insects in oilseed rape: implications for integrated pest management. – Agriculture, Ecosystems and Environment 95: 509-521.

Finch, S. & Skinner, G. 1976: The effect of plant density on populations of the cabbage root fly (*Erioschia brassicae* (Bch.)) and the cabbage stem weevil (*Ceutorhynchus quadridens* (Panz.)) on cauliflowers. – Bulletin of Entomological Research 66: 113-123.

Nitzsche, O. 1998: Auftreten und Effizienz von Parasitoiden als natürliche Gegenspieler von Schadinsekten im Winterraps unter besonderer Berücksichtigung unterschiedlicher Bodenbearbeitungsmaßnahmen nach Winterraps. – PhD Thesis, University of Göttingen.

Nuss, H. 2004: Effect of plant density and plant architecture on the abundance and within-plant distribution of stem borers in winter oilseed rape. – PhD Thesis, University of Göttingen, http://webdoc.sub.gwdg.de/diss/2004/nuss/nuss.pdf.

Powell,W., Walton, M.P. & Jervis, M.A. 1996: Populations and communities. – In: Jervis, M. & Kidd, N. (eds.). Insect natural enemies – practical approaches to their study and evaluation. Chapman & Hall, London: 223-292.

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weis, A.E. 1980: Interactions among three trophic levels: Influence of plants on interactions between insect herbivores and natural enemies. – Annual Review of Ecological Systematics 11: 41-65.

Ulber, B. 2003: Parasitoids of Ceutorhynchid stem weevils. In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Publishing, Oxford: 87-95.

Sauermann, W. & Gronow, J. 2000: Anbautechnik von Hybridraps. Ist eine sorten- und standortspezifische Intensität möglich? – Raps 18: 4-8.

Williams, I.H., Büchs, W., Hokkanen, H., Klukowski, Z., Luik, A,. Menzler-Hokkanen, I., Nilsson, C. & Ulber, B. 2004: The EU project MASTER (Management Strategies for European Rape pests): a review of progress. – IOBC/wprs Bulletin 27 (10): 3-16.

Larval parasitism of *Ceutorhynchus napi* Gyll. and *Ceutorhynchus pallidactylus* (Mrsh.) in plots of different crop density of oilseed rape

Kirsa Fischer and Bernd Ulber
Department of Crop Sciences, Plant Pathology and Plant Protection Division, Entomology Section, University of Goettingen, Grisebachstrasse 6, D-37077 Goettingen, Germany

Abstract: The rape stem weevil, *Ceutorhynchus napi* Gyll., and the cabbage stem weevil, *Ceutorhynchus pallidactylus* (Mrsh.), (Col., Curculionidae) are two of the most important stem-boring pests of oilseed rape in Germany. Natural enemies of *C. napi* and *C. pallidactylus* are the parasitic wasps *Tersilochus fulvipes* (Grav.) and *Tersilochus obscurator* Aub. (Hym., Ichneumonidae), respectively. The aim of this investigation was to examine the effect of crop density on the level of larval parasitism. Plant density may have an impact on plant architecture as well as on microclimate, thereby influencing the abundance and within-plant distribution of host larvae and hence the efficiency of parasitoids. In 2003/04 a field experiment was conducted including four sowing densities: 74, 49, 37 and 25 seeds/m^2. Various densities were achieved by choosing two row spacings (22.5 cm and 45 cm) and two intra row spacings (6 cm and 9 cm). The experiment was laid out in a randomized block design with four replications. Plant parameters recorded were length of the main raceme, diameter of the main raceme at its base, and at 50 cm and 2/3 along the raceme length, the number of leaves per plant and the number of lateral racemes. Samples of 20 plants per plot were analyzed in May to assess the abundance and within-plant distribution of the target pests *C. napi* and *C. pallidactylus* in the upper, lower and middle part of the stems as well as in the lateral racemes and in the leaves. The level of parasitism was detected by dissecting the larvae. In plots of 25 seeds/m^2 the plant length, the diameter of the main raceme, the number of leaves and the number of lateral racemes was significantly increased compared to plots of 74 seeds/m^2. The larval parasitism of *C. napi* was influenced by the sowing density as well. In the lower section of the main raceme, the level of parasitism was higher in plots of 74 seeds/m^2 than in the other treatments. Referring to the total number of larvae per plant, there was a tendency of decreasing levels of parasitism with lower sowing densities. The latter was also true for the level of parasitism in *C. pallidactylus*.

Pest damage to oilseeds – Techniques for developing solutions

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 205-211

Determining the sex of insect pests of oilseed rape for behavioural bioassays

Samantha M. Cook[1], Nigel P. Watts[1], Lynda M. Castle[2] and Ingrid H. Williams[1]
[1]*Plant & Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK;* [2]*Business & Information Services Division, Rothamsted Research, Harpenden, Herts., AL5 2JQ, UK.*

Abstract: Male and female insects of the same species often have different requirements for host plants, food resources and space. Therefore, insect responses to such stimuli in behavioural bioassays may vary according to the sex of the individuals tested. In order to compare differences in behavioural responses between female and male subjects, it is often necessary to determine the sex of an individual before its use in bioassays. Reliable techniques of determining the sex of live subjects which do not harm them are available for some species. We describe methods used to determine the sex of live, field-collected individuals of three major pest species of oilseed rape: pollen beetles (*Meligethes aeneus*), cabbage seed weevils (*Ceutorhynchus assimilis*) and cabbage stem flea beetles (*Psylliodes chrysocephala*).

Key words: Cabbage seed weevil, cabbage stem flea beetle, *Ceutorhynchus assimilis,* genitalia *Meligethes aeneus,* oilseed rape, pollen beetle, *Psylliodes chrysocephala,* sexual dimorphism.

Introduction

Yield potential in oilseed rape is reduced by damage caused by a wide variety of insect pests which infest the crop at different stages during its development (Alford *et al.*, 2003). These pests are currently controlled largely by synthetic pyrethroids. However, resistance of some pest populations to these insecticides has been reported (Hansen, 2003; Heimbach *et al.*, this issue; Wegorek, this issue); this resistance and concerns regarding operator safety, environmental pollution and negative effects on non-target organisms make finding alternative control strategies a research priority. The development of alternative control strategies requires an in-depth knowledge of the behavioural ecology of the pests and their natural enemies. This can be derived from detailed behavioural bioassays. Such bioassays may require the use of live individuals of known sex. For example, those which investigate responses between conspecifics (e.g. Cook *et al.*, this issue), including those relating to the identification of sex phermones (e.g. Bartlet *et al.*, 1994; Peng *et al.*, 1999; Bartelt *et al.*, 2001) or oviposition attractants/deterrents (e.g. Ferguson & Williams, 1991), those which compare the responses of males and females to experimental stimuli (Cook *et al.*, 2002a; Mauchline *et al.*, 2005) or those which simply aim to exclude interactions between males and females when groups of insects are tested (e.g. Cook *et al.*, 2002b).

The brassica pod midge *Dasineura brassicae* (Winnertz) (Diptera: Cecidomyiidae), and hymenopteran parasitoids of oilseed rape pests can be identified as female by their easily-visible ovipositors. However, other pest species, particularly the Coleopterans, do not possess such obvious sexual dimorphism. These insects can be tested individually and dissected after bioassay to determine their sex, but this is particularly time consuming and therefore costly, if large numbers of replicates need to be discarded because the wrong sex of insect was used.

Therefore, reliable techniques for determining the sex of live beetles prior to their use in bioassays are needed. These should not injure the beetle or affect its behaviour.

Here we describe methods enabling the sex determination of three important coleopteran pest species of oilseed rape: the pollen beetle, *Meligethes aeneus* (Fabricius) (Nitidulidae), the cabbage seed weevil, *Ceutoryhnchus assimilis* (Paykull) syn. *C. obstrictus* (Marsham) (Curculionidae) and the cabbage stem flea beetle *Psylliodes chrysocephala* (Linnaeus) (Chrysomelidae). These techniques have been developed to ensure that the sex of live individuals, can be easily determined without harming them.

Materials and methods

Pollen beetle (Meligethes aeneus)

Beetles for sex determination are confined in a plastic container (eg. 60-ml container, Bibby Sterilin Ltd, Stone, Staffs, UK, with an adapted, vented lid), and maintained on ice to slow their movement and prevent flight. The sex of each beetle is determined individually. A beetle is removed from the container using a fine paintbrush moistened with water. The beetle is then placed on its dorsal side on a glass microscope slide (75 x 25 mm) under view of a binocular microscope (eg. Wild MSA, Wild Heerbrugg, Switzerland) (x 12). A glass coverslip (22 x 22 mm) is placed over the beetle and gentle pressure is applied to its edge using the blunt wood end of the paintbrush. The pressure causes the genitalia to be extruded between the last visible sternite and last tergite. In females, the triangular-shaped ovipositor is extruded, whilst in males the U-shaped tegmen and aedeagus can be seen (Figure 1).

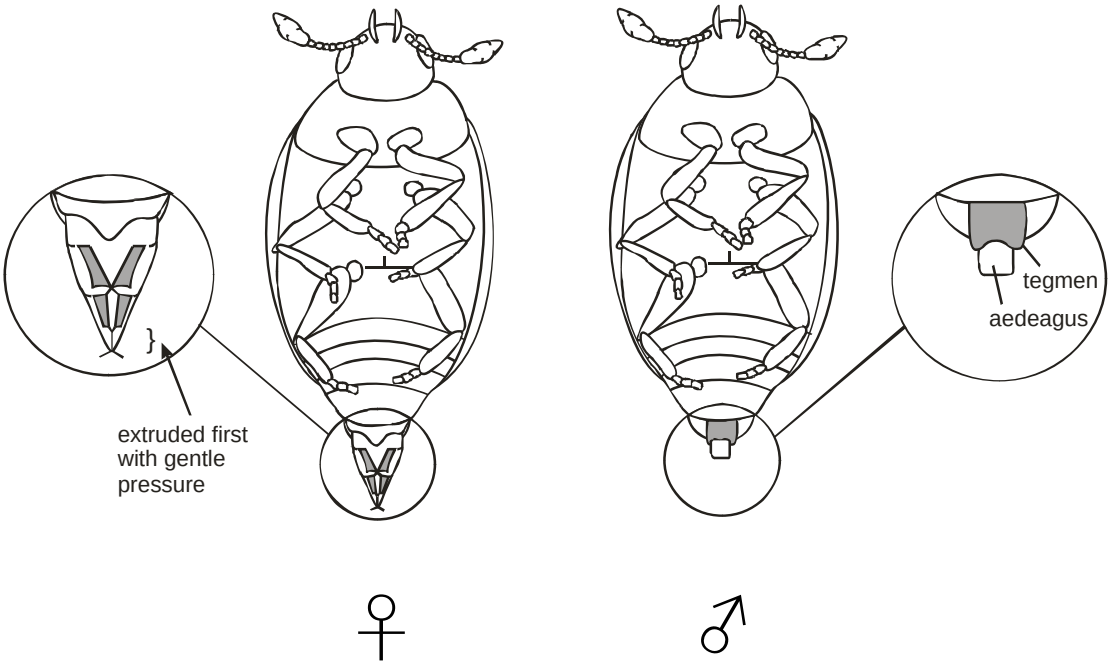


Figure 1. Sex determination of *Meligethes aeneus* adults by extrusion of genitalia under gentle pressure. Female with fully extruded ovipositor (left) and male showing extruded tegmen and aedeagus (right).

The amount of pressure required to reveal the genitalia varies from one beetle to the next; but generally, females extrude the ovipositior under less pressure than males extrude the aedeagus. When increasing pressure is applied to a female, the ovipositor is extruded to reveal

different sections. The first visible section is the ovipositor tip with styli (see Figure 1) which is sufficient to determine that the beetle is a female. Further pressure can be applied to reveal the second and third sections, but this increases the risk of injury. The male reveals the tegmen first, followed by the aedeagus with more pressure. Some males require excessive pressure to reveal the aedeagus which may result in injury and the beetle being discarded. Note that morphological characteristics of the ovipositor can be used to identify different species of *Meligethes* (detailed in Kirk-Spriggs, 1996).

Sex determination of pollen beetles using characteristics of the pupa has been described previously by Charpentier & Weibull (1994). This method is only suitable for individuals reared in the laboratory, and is impractical when large numbers of individuals are needed for bioassays. However, the method is reproduced here for completeness. The pupa is placed on its dorsal side and the ventral side is observed under a binocular microscope (x 12). The female possesses two 'dome-like projections' (tuberculae), visible between the 9th segment (pygidium) and the 8th abdominal sternite, which is absent in the male (Figure 2). In addition, the 8th sternite is narrower in the middle and more or less in two parts (biparate) in the male, but of uniform length and width in the female.

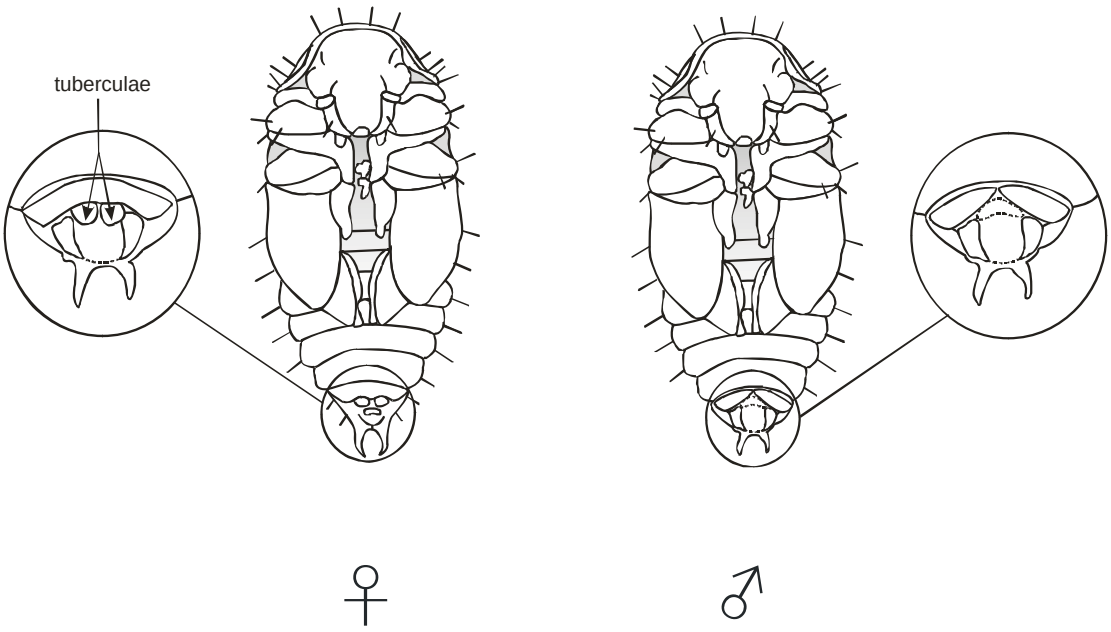


Figure 2. Sex determination of *Meligethes aeneus* pupae: female with two 'dome-shaped' tuberculae (left) which are absent in the male (right). After Charpentier & Weibull (1994).

Cabbage seed weevil (Ceutorhynchus assimilis)

Cabbage seed weevil sexes are determined by examining ventral features of the abdomen. A batch of seed weevils are placed in a plastic container with a vented lid, e.g. a 60-ml Sterilin container (as above), and maintained on ice. Sex determination is then carried out on a purpose-built **cold stage** which ensures that their body temperatures remain below their flight threshold temperature. The cold stage should be set at 0 ºC to slow movement down sufficiently to allow examination of their ventral features. In the absence of a cold stage, a plastic freezer block, used to chill food in cool boxes, could be used under a glass or metal

dish. The plastic freezer block would need changing regularly to maintain a low temperature. To improve conditions further, beetles could be examined in a cold room, e.g. 10 ºC.

The cold stage apparatus consists of a plastic water reservoir, a metal cold stage and a control unit. A water pump inside the reservoir circulates water in a closed system between the reservoir and the base of the cold stage. The latter contains electrical elements which heat or cool the metal plate and is controlled by the unit. The control unit has a digital display showing the current temperature of the cold plate and the temperature that has been set for it to reach. The water pump and control unit have independent mains plugs.

The cold plate is positioned under a binocular microscope (x 12) and 10 - 20 weevils are transferred onto it. A fibre-optic light source is positioned so that the light is focused from one side, casting a slight shadow from the weevils (note that the light should not be focused directly onto the weevils from above). This enables the features to be more easily distinguished. The weevils are positioned on their dorsal sides such that their ventral sides face upwards. The last abdominal sternite is examined to determine the sex. If the weevil has a wide concave depression it is a male (Figure 3). If it has a much narrower, shallower depression (sometimes visible as almost a 'slit' or a 'pinch'') the weevil is a female (Figure 3). Another distinguishing difference is the position on the rostrum where the scape sections of the antennae attach: in the male the scape end attaches to the middle of the rostrum; the scape end in the female attaches *above* the middle of the rostrum. However, this latter method requires closer examination of individuals than the former method, as the end of the scape can be difficult to see.

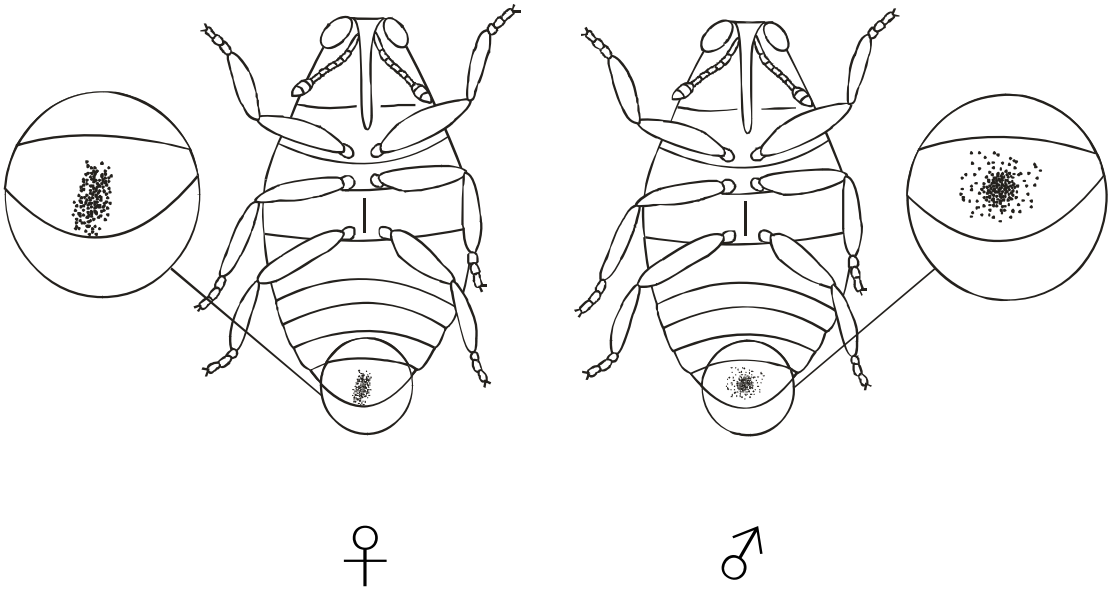


Figure 3. Sex determination of *Ceutorhynchus assimilis* adults using characteristics of the last abdominal sternite (ventral side). Males possess a large, deep, concave-shaped depression (right). Females possess a narrower depression often appearing as a 'slit' or 'pinch' (left).

Cabbage stem flea beetle (Psylliodes chrysocephala)

The sex of the cabbage stem flea beetle can be determined using characteristics on their tarsae. Groups of beetles (10-20) are placed in a Petri dish (Sterilin; 9 cm diameter) and the lid replaced. Due to the jumping ability of the beetle and to prevent escape, it is best to have the lid partly on the Petri dish, tilted up at one side, as the beetles are transferred into the dish

base. The dish is then positioned upside down (on the lid) under a binocular microscope (x 12) so the base faces upwards. The microscope is focused on the Petri dish base. As the beetles walk across the base with their ventral side facing upwards, their legs, are clearly visible through the microscope. In the male, the first tarsal segment on both the front and middle pairs of legs, is triangular-shaped and larger than the other segments of the tarsus (Figure 4). In the female the first tarsal segment is smaller than that in the male, and similar in size to the other segments (Figure 4). These features have previously been described and illustrated in Bonnemaison & Jourdeuil (1954), who describe the 1st tarsal segment of males and females as "heart shaped and as long as it is wide," and "cylindrical with a more or less constant diameter," respectively (in French).

After determining the sex of the beetle, it is carefully removed from the unsexed group using a fine artist's paintbrush (moistened). The Petri dish base is lifted up at one side and the paintbrush is pushed between the beetle's legs to encourage it to grip the paintbrush end. Several attempts may be necessary as the beetles are prone to jump when touched.

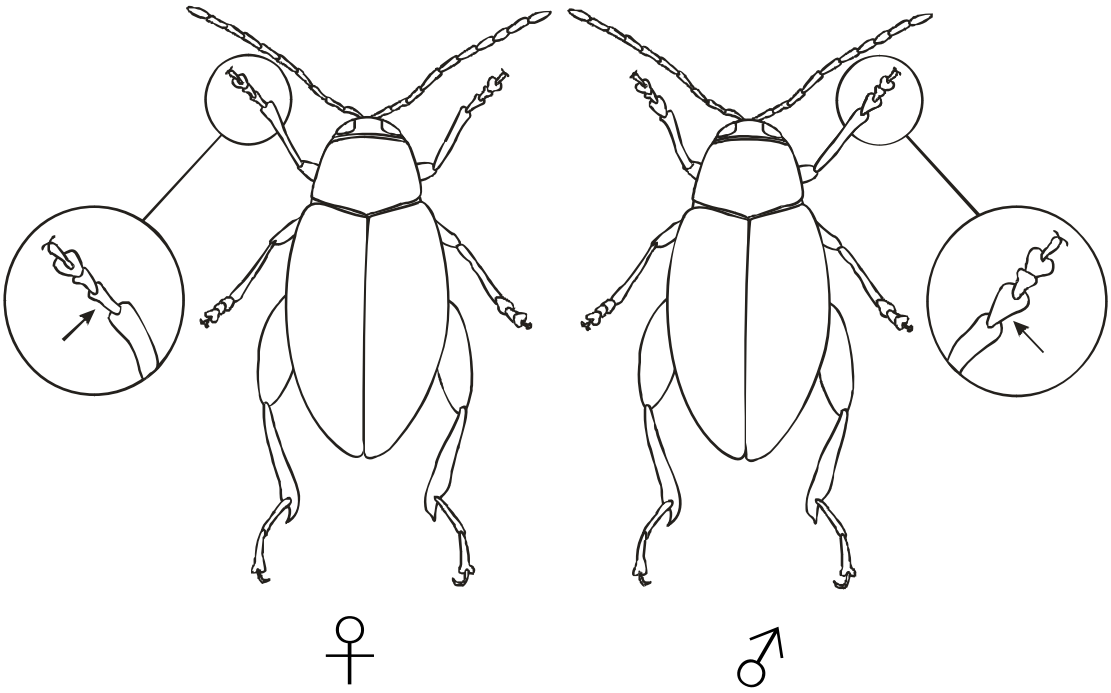


Figure 4. Sex determination of *Psylliodes chrysocephala* adults using features of the first tarsal segment of the front and middle pairs of legs. The first tarsal segment in the male is triangular-shaped and larger than other segments (right), this segment in females is smaller and more regular in size to the other segments (left). After Bonnemaison & Jourdeuil (1954).

Results and discussion

The accurate sex determination of live adult pollen beetles has previously been deemed impossible (Charpentier & Weibull, 1994; Borg & Ekbom, 1996). However, with care and practise, the technique described here has become routine in our laboratory, and is supported

by similar descriptions given by Ruther & Thiemann (1997). It has been used successfully in recent olfactometer studies to compare male and female responses to floral odours (Cook *et al.*, 2002a; Mauchline *et al.*, 2005). Likewise, the methods for determining the sexes of cabbage seed weevils and cabbage stem flea beetles have been verified as accurate and have been used to facilitate behavioural studies that would otherwise have been more difficult. Nazzi *et al.* (2001) used the method to obtain known male and female cabbage seed weevils from field-collected samples in order to test male olfactory responses to females. This enabled the existence and periodicity of a female-produced pheromone to be confirmed and determined. Bartlet *et al.* (1994) used the technique described here to determine the sexes of field-collected cabbage stem flea beetles and to investigate if male-specific antennal glands have a role in reproduction.

The techniques desribed here could be applied to determine the sexes of other coleopteran pests of oilseed rape in the same families as the species discussed in this paper. The stem-boring pests of oilseed rape, *Ceutorhynchus pallidactylus* (Marsham) syn. *C. quadridens* (Panzer) and *Ceutorhynchus picitarsis* Gyllenhal, (both Curculionidae) may share the morphological characteristics described for *C. assimilis*, thus enabling sex determination, but this needs to be determined and verified. LeSage & Paquin (1996) list external morphological differences between male and female *Aphthona* flea beetles (Chrysomelidae); differences between the male and female first tarsal segment are illustrated which are similar to the differences shown in this paper for *Psylliodes chrysocephala*. Peng *et al.* (1999) briefly discuss external morphological differences used in their study to determine the sexes of *Phyllotreta cruciferae* (Chrysomelidae); the shape of the abdomen tip was used, and is more rounded in males than females. Soroka *et al.* (2005) used pressure to extrude the genetalia of *P. cruciferae* flea beetles. Perhaps tarsal segment differences could also be used to determine the sex of *Phyllotreta*, as with *Aphthona* (LeSage & Paquin, 1992) and *P. chrysocephala* as described here, although this also remains to be determined and verified in future studies.

Acknowledgements

We thank Elspeth Bartlet and Andrew Ferguson for help in developing and verifying these methodologies. This work was undertaken on projects funded by the UK-Department for Food, Environment and Rural Affairs. Rothamsted Research receives grant-aided support from the UK Biotechnology and Biological Sciences Research Council.

References

Alford, D.V., Nilsson, C. & Ulber, B. 2003: Insect pests of oilseed rape crops. – In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Science, Oxford: 9-42.

Bartelt, R.J., Cosse, A.A., Zilkowski, B.W., Weisleder, D. & Momany, F.A. 2001: Male-specific sesquiterpenes from *Phyllotreta* and *Aphthona* flea beetles. – Journal of Chemical Ecology, 27: 2397-2423.

Bartlet, E., Isidoro, N. & Williams, I.H. 1994: Antennal glands in *Psylliodes chrysocephala*, and their possible role in reproductive behaviour. – Physiological Entomology, 19: 241-250.

Bonnemaison, L. & Jourdheuil, P. 1954: L'altise d'hiver du colza (*Psylliodes chrysocephala* L.). – Annales des Épiphyties, 5: 345-524.

Borg, A. & Ekbom, B. 1996: Characteristics of oviposition behavior of the pollen beetle, *Meligethes aeneus* on four different host plants. – Entomologia Experimentalis et Applicata, 81: 277-284.

Charpentier, R. & Weibull, J. 1994: Secondary sexual differences of pollen beetle, *Meligethes aeneus* Fabr. (Col., Nitidulidae) pupae. – Journal of Applied Entomology 118: 100-102.

Cook, S.M., Bartlet, E., Murray, D.A. & Williams, I.H. 2002a: The role of pollen odour in the attraction of pollen beetles to oilseed rape flowers. – Entomologia Experimentalis et Applicata 104: 43-50.

Cook, S.M., Murray, D.A., Watts, N.P. & Williams, I.H.: Responses of pollen beetles (*Meligethes aeneus*) to conspecific odours. – IOBC/wprs Bulletin 29(7): 143-150.

Cook, S.M., Smart, L.E., Potting, R.P.J., Bartlet, E., Martin, J.L., Murray, D.A., Watts, N.P. & Williams, I.H. 2002b: Turnip rape (*Brassica rapa*) as a trap crop to protect oilseed rape (*Brassica napus*) from infestation by insect pests: potential and mechanisms of action. – Proceedings of the BCPC Pests & Diseases conference, 18-21 November 2002, Brighton, UK: 569-574.

Ferguson, A.W. & Williams, I.H. 1991: Deposition and longevity of oviposition-deterring pheromone in the cabbage seed weevil. – Physiological Entomology 16: 27-33.

Hansen, L.M. 2003: Insecticide-resistant pollen beetles (*Meligethes aeneus* F) found in Danish oilseed rape (*Brassica napus* L) fields. – Pest Management Science 59: 1057-1059.

Heimbach, U., Müller, A. & Thieme, T.: First steps in analysing pyrethroid resistance of different oilseed rape pests in Germany. – IOBC/wprs Bulletin 29(7): 131-134.

Kirk-Spriggs, A.H. 1996: Pollen beetles. Coleoptera: Kateretidae and Nitidulidae: Meligethinae. – Royal Entomological Society of London, London.

LeSage, L. & Paquin, P. 1996: Identification keys for *Aphthona* flea beetles (Coleoptera: Chrysomelidae) introduced in Canada for the control of spurge (*Euphorbia* spp, Euphorbiaceae). – Canadian Entomologist 128: 593-603.

Mauchline, A.L., Osborne, J.L., Martin, A.P., Poppy, G.M. & Powell, W. 2005: The effects of non-host plant essential oil volatiles on the behaviour of the pollen beetle *Meligethes aeneus*. – Entomologia Experimentalis et Applicata 114: 181-188.

Nazzi, F., Bartlet, E., Watts, M. & Wadhams, L.J. 2001: Factors affecting the response of *Ceutorhynchus assimilis* Payk. (Col., Curculionidae) males to conspecific odour. – Journal of Applied Entomology 125: 433-435.

Peng, C.W., Bartelt, R.J. & Weiss, M.J. 1999: Male crucifer flea beetles produce an aggregation pheromone. Physiological Entomology 24: 98-99.

Ruther, J. & Thiemann, K. 1997: Response of the pollen beetle *Meligethes aeneus* to volatiles emitted by intact plants and conspecifics. – Entomologia Experimentalis et Applicata, 84: 183-188.

Soroka, J.J., Bartelt, R.J., Zilkowski, B.W. & Cosse, A.A. 2005: Responses of flea beetle *Phyllotreta cruciferae* to synthetic aggregation pheromone components and host plant volatiles in field trials. – Journal of Chemical Ecology 31: 1829-1843.

Wegorek, P.: Resistance of pollen beetle (*Meligethes aeneus* F.) to pyrethroids, chloronicotinyls and organophosphorous insecticides in Poland. – IOBC/wprs Bulletin 29(7): 135-140.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 213-221

Rearing and identification of the larval parasitoids of *Psylliodes chrysocephala* and *Ceutorhynchus pallidactylus* from field-collected specimens

Hassan Barari, Samantha M. Cook and Ingrid H. Williams
Plant & Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ. UK.

Abstract: *Psylliodes chrysocephala* (L.) (Coleoptera: Chrysomelidae) (cabbage stem flea beetle) and *Ceutorhynchus pallidactylus* (Marsh.) (Coleoptera: Curculionidae) (cabbage stem weevil) are two major stem-mining pests of oilseed rape (*Brassica napus*) in the UK. Little information is available on the occurrence, importance and efficiency of parasitoids in the biocontrol of these pests, due to sampling difficulties and inadequate knowledge on rearing and identification methods for the adults.

In this study, we developed an efficient method of rearing the larval parasitoids of *P. chrysocephala* and to a lesser extent, *C. pallidactylus* from field-collected specimens in order to identify the active larval parasitoids of these pests. Plant samples were collected in 2002 and 2003 from unsprayed winter oilseed rape crops at ~10-day intervals from mid-April until July. The samples were kept in cages in an outdoor insectary and the pest larvae were collected as they left naturally from the plant stems when ready to pupate. The larvae were identified to species and put into either 'pot emergence trap' or 'corked tube' containers containing sterilised soil, then kept under natural environmental conditions in the insectary. The adult pests were collected and counted as they emerged. The soil of the containers was then examined and parasitoid cocoons sorted from it, collected and counted. The cocoons were dissected and the pre-emergence adult parasitoids were removed and identified.

Adding 20% sharp sand to the soil increased the proportion of parasitoid cocoons found and reduced mortality in both containers. More parasitoid cocoons were found and lower mortality occurred in corked tubes than pot emergence traps in both years. These results suggest that the addition of sand to the soil and the use of corked tube containers contributed to the rearing success of the parasitoids by providing better conditions for their development. *Tersilochus microgaster* Szép. and *T. obscurator* Aub. (Hymenoptera: Ichneumonidae) were reared from *P. chrysocephala* and *C. pallidactylus* larvae, respectively. These parasitoids were identified for the first time in the UK as active parasitoids of these pests.

Key words: Rearing, parasitoid, oilseed rape, *Psylliodes chrysocephala, Ceutorhynchus pallidactylus*, *Tersilochus microgaster*, *Tersilochus obscurator*, stem-mining pest.

Introduction

Although there have been many studies on parasitoids of oilseed rape (*Brassica napus* L.) (Brassicaceae) pests, particularly in Europe, information on their rearing and identification is incomplete. This information is required for biocontrol studies because, in order to elucidate which parasitoids attack which hosts, it is necessary to collect the pest larvae in question, rear them in controlled conditions and identify the emerging adult parasitoids. Sampling, trapping and rearing methods for oilseed rape pests, and a new key to the identification of the hymenopterous parasitoids associated with oilseed rape pests have recently been published (Vidal, 2003; Williams *et al.*, 2003). However, as pointed out by these authors, there are still

many unresolved problems associated with the rearing and identification of the parasitoids of oilseed rape pests, particularly the stem-mining pests.

Psylliodes chrysocephala (L.) (Coleoptera: Chrysomelidae) (cabbage stem flea beetle) and *Ceutorhynchus pallidactylus* (Marsham), syn. *C. quadridens* (Panzer) (Coleoptera: Curculionidae) (cabbage stem weevil) are stem-mining pests of oilseed rape in the UK (Alford et. al., 2003). They are univoltine with three larval instars. The larvae feed inside the leaves (petioles and veins) and stem of the plant causing damage (Graham and Gould, 1980; Cox, 1998; Alford *et al.*, 2003).

The larvae of oilseed rape stem-mining pests are attacked by parasitoid species belonging to three hymenopteran families: Ichneumonidae, Braconidae and Pteromalidae. The main larval parasitoids belong to the genus *Tersilochus* Holmgren (Ichneumonidae: Tersilochinae) (Ulber, 2003; Ulber and Williams, 2003). *Tersilochus* parasitoids oviposit into their host larvae within plant petioles or stems in the spring. Although the parasitoid egg hatches within the host larva, most of the parasitoid's larval development occurs within the prepupal stage of the host in the soil in summer. The parasitoid larva spins a cocoon then pupates. The adult wasp remains in diapause inside the cocoon and emerges the following spring (Ulber, 2000).

In continental European studies the following Tersilochinae have been found to parasitize oilseed rape stem-mining pest larvae: *Tersilochus tripartitus* Brischk, which attacks the larvae of *P. chrysocephala* and *C. pallidactylus* (Jourdheuil, 1960), *Tersilochus microgaster* (Szépligeti) which attacks *P. chrysocephala* larvae (Nitzsche, 1998), and *Tersilochus obscurator* Aubert and *Tersilochus exilis* Holmgren which both attack larvae of *C. pallidactylus* (Jourdheuil, 1960; Herrström, 1964; Ulber, 2000). However, it is not known which of these species are active parasitoids in the UK. One potential reason for the scarcity of information on parasitoids of stem-mining pests of oilseed rape is the difficulty in collecting adult parasitoids of the pests in the field. The phenology and emergence time of the parasitoids is little understood, so finding the correct time for sampling is difficult. In addition, the adult parasitoids usually occur at a low level within the plant canopy, where they search for stem-mining pest larvae (Barari et al., unpublished data) hence, collecting them by sweep-netting is not feasible. Therefore, to collect adult parasitoids and to ensure that they are active parasitoids of *P. chrysocephala* or *C. pallidactylus* larvae, we reared the larval parasitoids of the pests from field-collected hosts.

In continental Europe, there have been a few investigations on rearing the larval parasitoids of *P. chrysocephala* and *C. pallidactylus* infesting oilseed rape (Klingenberg and Ulber, 1994; Klukowski and Kelm, 2000; Kraus and Kromp, 2002). In all three studies, the pest larvae were dissected from plants collected in the field and then reared in plastic boxes containing sterilised moistened soil for pupation. However, Klingenberg and Ulber (1994) found that the parasitism rate of the fully developed pest larvae that leave the plant stems naturally when ready to pupate is higher than those collected through plant dissection. Therefore, to improve our chances of obtaining parasitized larvae, we aimed to develop more natural methods than used in previous studies to rear larval parasitoids of *P. chrysocephala* and *C. pallidactylus* in order to identify the active larval parasitoids of these pests in the UK.

Materials and methods

Plant sample collection

Oilseed rape (*B. napus* and *Brassica rapa*) stems damaged by the pest larvae were collected in 2002 and 2003 from crops that had not been treated with insecticide, grown on Rothamsted Farm, Harpenden, Hertfordshire, UK., at approximately 10-day intervals from mid-April until the crop was desiccated in July. This time period covered the whole period of adult parasitoid

activity in the field (Barari *et al*., unpublished). Plants showing symptoms of stem-mining pest damage (exit holes on stems and visible tunnelling damage to stems and petioles) were collected. The buds, flowers and pods were removed from the plants in the field to eliminate the larvae of other coleopteran pests e.g. cabbage seed weevil, *Ceutorhynchus assimilis*; only the stems were kept to capture the mature stem-mining pest larvae as they exited the stems, ready to pupate.

Pest larvae collection

The plant samples were taken to an outdoor insectary and kept in cages for one week. The insectary was protected from direct sunlight and had two sides open to the outside that were covered with net. Six cages were used to collect the pest larvae from plant samples. Each cage was 160 cm high, 50 cm long and 50 cm wide and had net sides with a plexiglass door. The cage had a drawer underneath which was separated from the main part of the cage by a metal grid. Mature larvae ready to pupate exited the stems and dropped down into the drawers. These were covered on the inside with a black plastic sheet to make the pest larvae more visible. The drawers were checked daily and any larvae removed using a moistened paint brush. They were identified to species (*P. chrysocephala* or *C. pallidactylus*) using the key by Alford *et al*. (2003) and put into either 'pot emergence trap' or 'corked tube' containers.

The pot emergence traps (Figure 1) were each made of a plastic flower pot (13.5 cm diameter, 12.5 cm deep), on the top of which was a metal frame (14 cm height) topped with a plastic end screw connector. The frames and the top of the flower pot were covered with black tulle to prevent any emerging insect from escaping. Emerged insects were collected from a transparent inverted plastic container (8.5 cm diameter, 10.5 cm deep) with a screw-on lid. An open-ended sterilin tube connected the container via a screw end to the top of the metal frame.

The corked tubes (Figure 2) were glass specimen tubes (2.4 cm diameter, 7.2 cm deep) with cork stoppers (VWR International Ltd., Lutterworth, Leicestershire, UK). Both pot emergence traps and corked tubes were half-filled with soil (collected from an oilseed rape field that had not been treated with insecticide, sifted, mixed with 20% sharp sand (in 2003), and sterilised at 120° C for 20 minutes). The soil in the pot emergence traps was covered with moss to maintain humidity. Between 5 – 20 and 1 – 5 larvae of the same species were put in each pot emergence trap and corked tube, respectively. Larvae were placed on the soil surface and were allowed to bury themselves in the soil. The pot emergence traps and corked tubes containing the pest larvae were then left in the insectary on a shelf out of direct sunlight. The pot emergence traps were checked weekly and mist-sprayed with tap water if they appeared dry. Such treatment was unnecessary with the corked tubes, as the cork maintained the moisture conditions within the tubes.

Parasitoid collection

Once the first adult pests had emerged and were captured in the inverted plastic containers of the pot emergence traps or were seen inside the corked tubes, the soil was emptied into a white plastic tray placed under a magnifying glass and was carefully sorted using a fine paint brush and forceps. Any parasitoid cocoons found were collected. The cocoons (Figure 3) were put into a corked tube without any soil and left in the insectary for one month to allow the parasitoids to fully develop. The cocoons were then dissected under a binocular microscope (x 6) and pre-emergence parasitoids (Figure 4) were removed and transferred into 100% ethanol for storage at 5° C before later identification.

Parasitoid identification

The female parasitoids that emerged from *P. chrysocephala* and *C. pallidactylus* larvae were identified using keys by Vidal (2003) and Ferguson's 'A quick key to the identification of Tersilochinae parasitic on insect pests of oilseed rape' (A.W. Ferguson, unpublished). Two

morphological characters described in the keys were primarily used to separate the species of larval endoparasitoids of *P. chrysocephala* and *C. pallidactylus* belonging to Tersilochinae (Hymenoptera: Ichneumonidae): the length of sternaulus (a curved furrow dividing the lower part of the mesopleuron), and the proportion of the ovipositor sheath length to the length of petiolar tergite (tergite of first metasomal segment) (Figure 5).

Figure 1. Pot emergence trap

Figure 2. Corked tubes

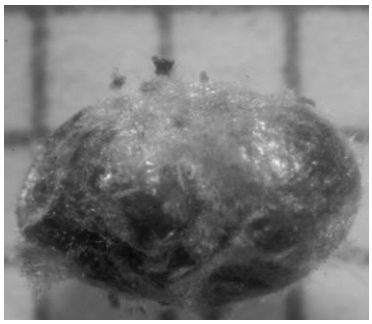


Figure 3. *Tersilochus obscurator* cocoon Background grid is 1 mm

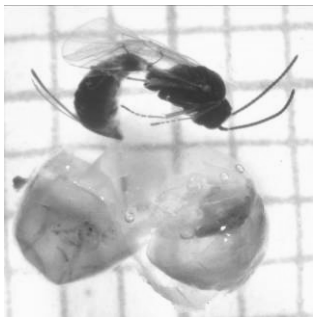


Figure 4. Pre-emergence *Tersilochus obscurator* ♀ removed from its cocoon. Background grid is 1 mm

Results

Rearing and identifying parasitoids of* Psylliodes chrysocephala *larvae

In 2002, between 29 April and 7 July, a total of 465 mature *P. chrysocephala* larvae were collected after their emergence from infested plant stems; 348 were reared in pot emergence traps and 117 in corked tubes (Table 1). Overall, 60 (13%) adult beetles emerged during summer; 10.9% in the pot emergence traps and 18.8% in corked tubes (Table 1). The first beetle emerged in a corked tube on 20 June; 23 days after placing the larva onto the sterilised soil. After sorting the soil of the pot emergence traps and corked tubes, 23 parasitoid cocoons were found (5% of total; 15 in the pot emergence traps and 8 in corked tubes) (Table 1). Two of these were put in corked tubes containing sterilised soil, and left in the insectary to emerge naturally. The remaining 21 cocoons were dissected and 21 (15♀, 6♂) adult pre-emergence larval parasitoids of *P. chrysocephala* were removed. An adult male parasitoid emerged on 24 March 2003 from one of the parasitoid cocoons left in the insectary. The other did not emerge. Overall, mortality was 82.2%; 84.8% and 74.4% in the pot emergence traps and corked tubes, respectively (Table 1).

In 2003, 2991 *P. chrysocephala* larvae were collected between 23 April and 18 June (Table 1). In total, 1861 (62%) adult beetles emerged during summer; 28.7% in pot emergence traps and 68.1% in corked tubes (Table 1). The first adult beetle emerged from a corked tube on 13 June; 45 days after placing the larva on the soil. Thereafter, 383 parasitoid cocoons were found in the soil (12.8% of total; 21 in the pot emergence traps and 362 in corked tubes). All these were dissected and 383 (191 ♀, 192 ♂) adult pre-emergence larval parasitoids of *P. chrysocephala* were removed (Table 1). *Psylliodes chrysocephala* larval mortality in pot emergence traps and corked tubes was 66.6% and 17.7%, respectively (Table1); total mortality was 25%.

The 206 female adult parasitoids of *P. chrysocephala* larvae collected from the rearing procedures in both years were all identified as *Tersilochus microgaster* (Szépligeti) (Hymenoptera: Ichneumonidae: Tersilochinae). The identity was confirmed by comparison with voucher specimens held at Rothamsted Research by A. W. Ferguson.

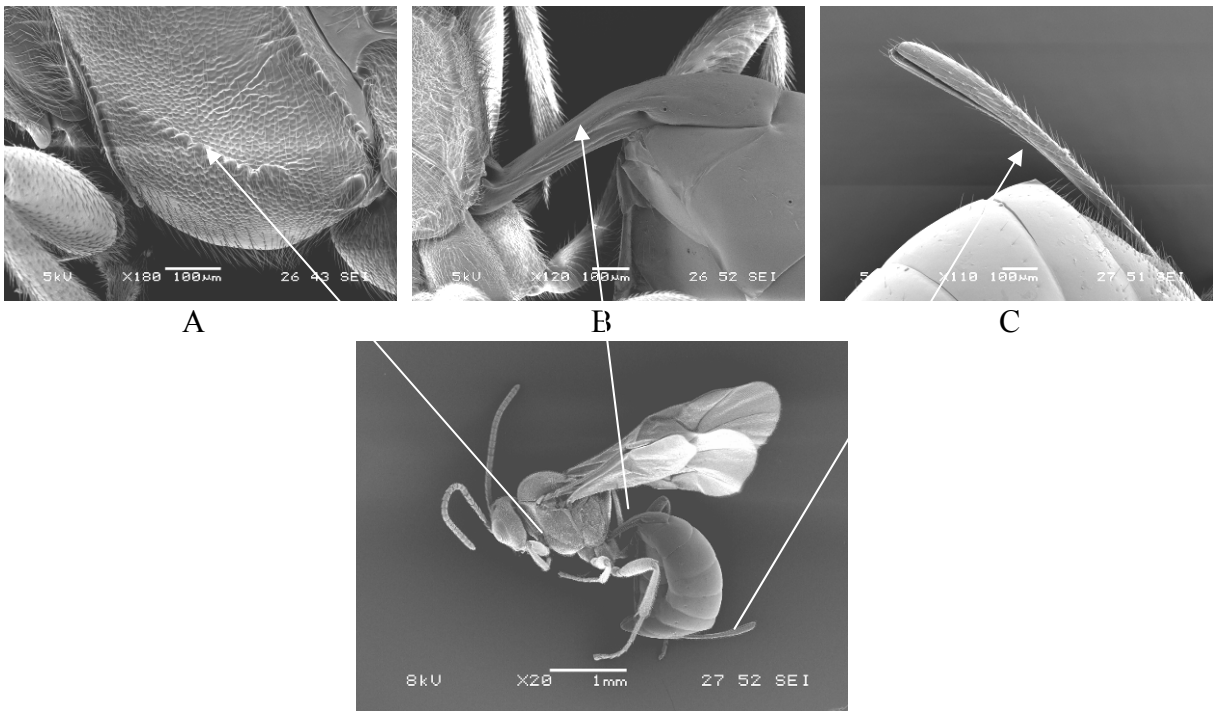


Figure 5. Scanning electron micrograph of morphological characters of *Tersilochus microgaster*, which are used to separate this species from *T. obscurator*
A) Sternaulus, B) Petiole, C) Ovipositor sheaths

Rearing and identifying parasitoids of* Ceutorhynchus pallidactylus *larvae

In 2002, from 31 May until 29 July, a total of 192 *C. pallidactylus* larvae were collected after they dropped from the damaged plants, and 144 were reared in pot emergence traps and 48 in corked tubes (Table 1). Overall, 30 (15.6%) adult beetles emerged during summer; 15.3% in the pot emergence traps and 16.7% in corked tubes (Table 1). The first beetle emerged in a corked tube on 4 July 2002; one month after the larva was placed onto soil. By sorting the soil of pot emergence traps and corked tubes, ten parasitoid cocoons were removed (5% of total; 7 in the pot emergence traps and 3 in corked tubes) (Table 1). Two of these were put in a corked tube containing sterilised soil, and left in the insectary to emerge naturally, and the remaining eight cocoons were dissected. Eight (3♀ and 5♂) adult pre-emergence larval parasitoids of *C.*

pallidactylus were removed (Table 1). Adult male parasitoids emerged naturally from both cocoons left in the soil in the insectary on 28 February 2003 and 5 March 2003. Larval mortality in pot emergence traps and corked tubes was 79.9% and 77.1%, respectively (Table 1); total mortality was 79.2%.

Table 1. Number of pest larvae (*Psylliodes chrysocephla* and *Ceutorhynchus pallidactylus*) reared in the pot emergence trap (PET) and corked tube (CT) containers, the percentage of adult pests emerged and their parasitoids collected through rearing parasitoids in the insectary in 2002 and 2003.

Pest	Year	No. pest larvae in soil		% adult pests emerged		% parasitoid cocoons found		% mortality		No. adult parasitoids reared	
		PET	CT	PET	CT	PET	CT	PET	CT	PET	CT
P. chrysocephala	2002	348	117	10.9	18.8	4.3	6.8	84.8	74.4	3♂ 12♀	4♂ 3♀ †
	2003	446	2545	28.7	68.1	4.7	14.2	66.6	17.7	9♂ 12♀	183♂ 179♀
C. pallidactylus	2002	144	48	15.3	16.7	4.9	6.2	79.9	77.1	4♂ 1♀	3♂ 2♀ ††
	2003	-	102	-	7.8	-	9.8	-	82.3	-	6♂ 4♀

† Two parasitoids were withheld to emerge naturally; 1 male emerged, the other did not.
†† Two parasitoids were withheld to emerge naturally; 2 males emerged.

In 2003, from 14 June until 11 July, 102 *C. pallidactylus* larvae were collected (Table 1) and all were reared in corked tubes. Eight (7.8%) adult weevils emerged during summer. The first adult weevil emerged on 12 July; 28 days after placing the larva onto the soil. Thereafter, 10 (9.8%) parasitoid cocoons were found in the soil. These were dissected and 10 (6♀, 4♂) adult pre-emergence larval parasitoids of *C. pallidactylus* were removed (Table 1).

The nine female adult parasitoids of *C. pallidactylus* larvae collected from the rearing procedures in 2002 and 2003 were all identified as *Tersilochus obscurator* Aubert (Hymenoptera: Ichneumonidae: Tersilochinae). The identity was confirmed by comparison with voucher specimens held at Rothamsted Research by A.W. Ferguson.

Discussion

Parasitoid rearing

Rearing the larval endoparasitoids of the stem-mining pests of oilseed rape, *P. chrysocephala* and *C. pallidactylus*, was more successful using the corked tube than the pot emergence trap containers. More parasitoid cocoons were found and mortality was lower in the corked tubes than in the pot emergence traps in both years of this study (Table 1). *Psylliodes chrysocephala* larval mortality was reduced from 82% in 2002 to 25% in 2003, and mortality in the corked tubes was 3.8 times lower than that in pot emergence traps (Table 1). These results suggest that corked tubes are more productive containers than pot emergence traps, possibly due to better water regulation properties of the former compared with the latter (see below). The number of *C. pallidactylus* larvae collected was low, but the percentage of parasitoid cocoons found was comparable to *P. chrysocephala*. Therefore, in future studies more plant samples need to be collected to evaluate the efficiency of this rearing method for *C. pallidactylus*.

Mortality of the pest larvae in the soil was high, particularly in 2002 (in total, 82.2% *P. chrysocephala* and 79.2% *C. pallidactylus*). According to our observations during sorting through the soil, it seems there were at least two reasons for this mortality. The soil in many containers was too heavy and compacted and in some pot emergence traps was too wet. These conditions may have made the soil unsuitable for the larval/pupal development. These problems were resolved, to some extent, in 2003 by adding 20% sharp sand to the soil. This vastly improved success of rearing both adult pests and adult parasitoids from their larvae, particularly in the corked tubes.

Rearing parasitoid larvae to adult from mature host larvae has several advantages. In this study, the plant samples were collected at approximately 10-day intervals, and the pest larvae collected were mature and had left the host plant stems naturally. Such full-term larvae were more likely to be parasitized than those collected prematurely through plant dissection and extraction (Klingenberg and Ulber, 1994). The pest larvae were reared thereafter under natural environmental conditions (temperature and light regimes). Hence, the life history and phenological development of the pests and their parasitoids during our rearing procedures were more likely to be similar to that in the field than rearing in unnatural conditions (Shaw, 1997). During this investigation, both *P. chrysocephala* and *C. pallidactylus* third instar larvae were found to leave the plants and drop into the soil for pupation only during the night, because no pest larvae were observed in the drawers of cages during the day. The observed dates of emergence concur with previous studies; *P. chrysocephala* adults started to emerge in mid June (13-20) and *C. pallidactylus* adults emerged in early July (4-12). In field conditions, new generation adults of *P. chrysocephala* and *C. pallidactylus* were collected in emergence traps from 27 June onwards (Ferguson *et al.*, 2003). Also, by allowing some of the parasitoids to emerge naturally from the cocoons, we found that adult *T. microgaster* emerged on 24 March: a timing supported by Nitzsche (1998, cited by Ulber & Williams (2003)). The adult *T. obscurator* emerged at the end of February and the beginning of March. However, according to Ulber (2003), this parasitoid colonised oilseed rape in April or May, when the early larval instars of cabbage stem weevil occur within petioles.

Parasitoid identification

This study has confirmed, for the first time, that *T. microgaster* and *T. obscurator* are larval endoparasitoids of *P. chrysocephala* and *C. pallidactylus* , respectively in the UK.

The occurrence of *T. microgaster* in water traps placed in oilseed rape fields was reported for first time in the UK by Barari et al., (submitted). Here we confirm that this species is an active parasitoid of *P. chrysocephala* larvae in the UK by rearing it from its host. This species is a solitary endoparasitoid and has one generation per year (Ulber and Williams, 2003). It has also been reared from *P. chrysocephala* larvae in Germany (Klingenberg and Ulber, 1994), but its distribution elsewhere is unknown.

Tersilochus obscurator was reared from *C. pallidactylus* larvae. Here this species is confirmed as an active parasitoid of *C. pallidactylus* larvae in the UK by rearing it from its host. It is a solitary endoparasitoid and the most widespread and abundant parasitoid of *C. pallidactylus* larvae in Europe; reported from France (Jourdheuil, 1960), Germany (Lehmann, 1965; Klingenberg and Ulber, 1994; Nissen, 1997; Nitzsche, 1998), former Czechoslovakia (Šedivy, 1983), and the UK, Hungary, Ireland, Sweden and the Ukraine (Horstmann, 1981).

We found that identification of some *Tersilochus* species using only their morphological characters to be problematic. According to the latest identification keys (Vidal, (2003) and A. W. Ferguson (unpublished)), *T. microgaster* is separated from *T. obscurator* mainly by differences in the proportion of the ovipositor sheath to the length of the petiolar tergite, and the percentage of mesopleuron length spanned by the length of the sternaulus. In *T. microgaster*, ovipositor sheaths are ≤1.4 x the length of petiolar tergite, and the sternaulus is

defined by a series of pits extending over 50-80% of the length of the mesopleuron. In *T. obscurator*, ovipositor sheaths are ≥1.4 x the length of the petiolar tergite, and the sternaulus is weakly defined by a series of pits and extending up to half of the length of the mesopleuron. However, in this study, some (3% n=162) adult *Tersilochus* reared from *P. chrysocephala* larvae had morphological measurements exceeding this range; the proportion of the ovipositor sheath to the petiolar tergite ranged between 1.40-1.48, and in some specimens the sternaulus spanned more than 80% of the mesopleuron length. Molecular techniques using DNA sequencing are therefore being developed to aid the identification and separation of these *Tersilochus* species.

Acknowledgements

We thank Andrew Ferguson for help in parasitoid identification and Mark Shaw for advice on rearing methodology. Andrew Ferguson and Adrian Hobbs designed the pot emergence traps. This work was partly-funded by the UK Department of Environment, Food and Rural Affairs and contributed to the EU project MASTER (QLK5-CT-2001–01447). The Iranian Government funded a scholarship for Hassan Barari. Rothamsted Research receives grant-aided support from the UK Biotechnology and Biological Sciences Research Council.

References

Alford, D.V., Nilsson, C. & Ulber, B. 2003: Insect pests of oilseed rape crops. – In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Science, Oxford, UK: 9-41.

Cox, M.L. 1998: The genus *Psylliodes* Latreille (Chrysomelidae: Alticinae) in the UK. – Coleopterist 2: 33-65.

Ferguson, A.W., Klukowski, Z., Walczak, B., Clark, S.J., Mugglestone, M., Perry, J.N. & Williams, I.H. 2003: Spatial distribution of pest insects in oilseed rape: implications for integrated pest management. – Agric., Ecosys. and Environ. 95: 509-521.

Graham, C.W. & Gould, H.J. 1980: Cabbage stem weevil (*Ceutorhynchus quadridens*) on spring oilseed rape in southern England and its control. – Ann. Appl. Biol. 95: 1-10.

Herrström, G. 1964: Untersuchungen über Parasiten von Ölfruchtschädlingen in Schweden. – Statens växtskyddsanstalt, Meddelanden 12: 437-448.

Horstmann, K. 1981: Revision der europäischen Tersilochinae II (Hymenoptera, Ichneumonidae). – Spixiana, Supplementum 4: 1-76.

Jourdheuil, P. 1960: Influence de quelques facteurs écologigues sur les fluctuations de population d'une biocénose parasitaire: étude relative à quelqes hyménoptères (Ophioninae, Diospilinae, Euphorinae) parasites de divers Coléoptères infeodes aux Crucifères. – Annals Épiphyt. 11: 445-658.

Klingenberg, A. & Ulber, B. 1994: Investigations on the occurrence of Tersilochinae (Hym, Ichneumonidae) as parasitoids of oilseed rape pests in the Göttingen region in 1990 and 1991, and on their emergence following various tillage techniques. – Journal of Applied Entomology 117: 287-299.

Klukowski, Z. & Kelm, M. 2000: *Stenomalina gracilis* (Walker), a new parasitoid reared from *Ceutorhynchus napi* Gyll. in Poland. – IOBC/wprs Bull. 23(6): 135-138.

Kraus, P. & Kromp, B. 2002: Parasitization rates of the oilseed rape pests *Ceutorhynchus napi*, *Ceutorhynchus pallidactylus* (Coleoptera, Curculionidae) and *Meligethes aeneus* (Coleoptera, Nitidulidae) by ichneumonids in several localities on Eastern Austria. – IOBC/wprs Bull. 25 (2): 117-122.

Lehmann, W. 1965: Einfluß chemischer Bekämpfungsmaßnahmen auf einige Rapsschädlinge und ihre Parasiten. II. Knospen- und Stengelschädlinge. – Archiv für Pflanzenschutz 1: 209-219.

Nissen, U. 1997: Ökologische Studien zum Auftreten von Schadinsekten und ihren Parasitoiden an Winterraps norddeutscher Anbaugebiete. – PhD Thesis. Christian-Albrechts-University of Kiel.

Nitzsche, O. 1998. Auftreten und Effizienz von Parasitoiden als natürliche Gegenspieler von Schadinsekten im Winterraps unter besonderer Berücksichtigung unterschiedliedlicher Bodenbearbeitungsmaßnahmen nach Winterraps. – PhD Thesis. Georg-August-University of Göttingen.

Šedivy, J. 1983: Tersilochinae as parasitoids of insect pests of winter rape (Hymenoptera, Ichneumonidae). – Contribution of the American Entomological Institute 20: 266-276.

Shaw, M.R. 1997: Rearing parasitic Hymenoptera. – The Amateur Entomologist 25: 1-46.

Ulber, B. 2000: Bibliography of parasitoid species and levels of parasitism of rape stem weevil, *Ceutorhynchus napi* Gyll., and cabbage stem weevil, *Ceutorhynchus pallidactylus* (Marsh.). –IOBC/wprs Bull. 23: (6): 131-134.

Ulber, B. 2003: Parasitoids of ceutorhynchid stem weevils. – In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Science, Oxford, UK: 87-95.

Ulber, B. & Williams, I.H. 2003: Parasitoids of flea beetles. – In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Science, Oxford, UK: 125-138.

Vidal, S. 2003: Identification of hymenopterous parasitoids associated with oilseed rape pests. – In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Science, Oxford, UK: 161-179.

Williams, I.H., Büchi, R. & Ulber, B. 2003: Sampling, trapping and rearing the pests of oilseed rape and their parasitoids. – In: Alford, D.V. (ed.). Bio-control of oilseed rape pests Blackwell Science, Oxford, UK: 145-160.

PATHOLOGY PAPERS

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 225-227

OREGIN collection of oilseed rape fungal pathogen isolates managed by a relational database accessible to stakeholders via the Internet

Akinwunmi O. Latunde-Dada, Paul E. Hornby, Nathalie Castells-Brooke, Neal Evans, Bruce D.L. Fitt and Graham J. King
Rothamsted Research, Harpenden, Herts, AL5 2JQ, U.K.

Abstract: One aim of the Defra-funded Oilseed Rape Genetic Improvement Network (OREGIN) is to establish, characterise and catalogue a collection of *Leptosphaeria maculans* (phoma stem canker) and *Pyrenopeziza brassicae* (light leaf spot) isolates from around the world and to determine genetic diversity in the world-wide populations of these pathogens. Five hundred and twenty isolates of *L. maculans* and 253 isolates of *P. brassicae* have been assembled at Rothamsted Research from sources representative of the world-wide distribution of phoma stem canker and light leaf spot. Information on these isolates (including geographical origin, host species and cultivar of origin, pathogenicity group, mating type and other properties such as culture medium, stock type [slope culture/ glycerol spore suspension stocks]) is now being collected and saved to a Rothamsted Research MySQL open source relational database server. This is being made accessible to the research community and stakeholders via the OREGIN website (www.oregin.info). Once characterised and described, the isolates will be available and the website will generate the appropriate Material Transfer Agreement (MTA) forms. The OREGIN website is being expanded to include access to information relating to other public domain plant resources being made available via OREGIN. This includes information about the *Brassica napus* Diversity Fixed Foundation Set (BnDFFS) and reference genetic mapping populations that are held in secure storage at Warwick HRI.

Keywords: *Brassica napus*, diversity, *Leptosphaeria maculans*, MySQL, pathogen collection, PHP, *Pyrenopezia brassicae*, resistance, slope, spore.

Introduction

Oilseed rape is currently the most profitable arable crop in the UK and is important for edible oil, animal feed and in projections for biodiesel production. However, it is produced under high-input conditions (fertiliser, herbicide, insecticide and fungicide) with the concomitant negative environmental footprints. The Defra-funded *O*ilseed *R*ap*e* *G*enetic *I*mprovement *N*etwork (OREGIN) was established to reduce reliance on high-input agriculture while maintaining, via genetic improvement, the high and sustainable yields required by growers. Crop loss due to phoma stem canker (caused by *Leptosphaeria maculans*) and light leaf spot (caused by *Pyrenopeziza brassicae*) amounts to about £50M annually and ranks very highly amongst problems associated with oilseed rape production. OREGIN is developing, for screening and selection, a set of genetically fixed lines representing a structured and objective sampling of diversity within the worldwide *B. napus* genepool. To enable a meaningful screening of these lines for components of durable disease resistance, a culture collection of isolates of the two fungal pathogens obtained from geographically diverse sources is being assembled at Rothamsted Research. This paper reports the establishment of the OREGIN culture collection, currently comprising 520 isolates of *L.maculans* and 253 isolates of *P. brassicae*, and the development of a relational database to make information on these pathogens publicly available to stakeholders.

The Culture Collection

The OREGIN culture collection (Table 1) has been assembled from isolates of both pathogens held originally at Rothamsted Research, as well as fresh acquisitions from researchers and laboratories world-wide. Isolates of *L. maculans* are grown (15-25°C) on potato dextrose agar plates and slopes; those of *P. brassicae* are grown (15°C) on malt extract agar plates and slopes. Long-term cultures are kept in sealed plates and slopes at 4°C. In addition, conidial suspensions of the pathogens are kept in 10% glycerol at -80°C and securely held in duplicates at 2 separate locations. Some of the historical isolates in the OREGIN culture collection have been cited in peer-reviewed papers published in scientific journals (Purwantara *et al*., 2000; Balesdent *et al*., 2005; Simons and Skidmore, 1988; Ilott *et al*., 1984; Majer *et al*., 1996). Genomic DNA extracted from mycelial cultures of isolates in the collection will be stored at -20 ^{o}C and all isolates will be authenticated by PCR diagnostics

Table 1. The OREGIN pathogen culture collection: current geographical and species composition

Country of origin	**Isolates per country**	***Leptosphaeria maculans***	***Pyrenopeziza brassicae***
Australia	141	141	0
Austria	13	13	0
Brazil	5	5	0
Canada	29	29	0
France	158	43	115
Germany	78	73	5
Mexico	18	18	0
Poland	52	48	4
Portugal	9	9	0
Sweden	20	20	0
United Kingdom	238	109	129
United States of America	12	12	0
Total	**773**	**520**	**253**

The Database

For the purpose of data management and future communication with widely distributed stakeholders, a website-accessible relational database server was developed and has now reached the stage that collection curation and other stringent quality assurance requirements at Rothamsted Research can be done on-line periodically. Technical expertise was provided by the resident website developer at Rothamsted with experience in MySQL database design and the PHP scripting language. Security and membership functions have been programmed into parts of the website, particularly to prevent unauthorised data modification. The database is easily accessible but permissions are required to add, delete or edit data. Users are encouraged to register. The OREGIN database menu can be accessed by clicking on the resources tab on the OREGIN website.

The Future

Future database work will involve adding and updating data, improving the search capabilities to allow stakeholders and colleagues to view isolates according to their chosen search criteria. The ability to order isolates from the collection and to automatically generate the necessary material transfer agreements (MTAs) will be programmed into the website too. A nominal fee to cover materials and postage may be charged.

The *Brassica napus* Diversity Fixed Foundation Set (BnDFFS) database of plant resources, currently maintained on a separate server, will be seamlessly integrated into the OREGIN website and links to *Brassica napus* genetic maps and genomic data will be made. Information will be made available on a genetically diverse subset of isolates from the OREGIN culture collection that will be used in experiments to screen lines of the BnDFFS (developed at WHRI and currently being multiplied) for resistance to stem canker and light leaf spot.

Acknowledgements

We thank the following for the donation of isolates from the countries indicated in parentheses: Ingrid Happstadius (Sweden), Zbigniew Karolewski and Malgorzata Jedryczka (Poland), Martin Barbetti and Barbara Howlett (Australia), Marie-Helene Balesdent and Thierry Rouxel (France, Portugal), Roger Rimmer (Canada), Birger Koopman (Germany). The OREGIN project is funded by the Department for Environment, Food and Rural Affairs, UK. Rothamsted Research receives funding through the UK Biotechnology and Biological Sciences Research Council.

References

Balesdent, M.H., Barbetti, M.J., Li, H., Sivasithamparan, K., Gout, L. & Rouxel, T. (2005). Analysis of *Leptosphaeria maculans* race structure in a worldwide collection of isolates. – Phytopathology 95: 1061-1071.

Ilott, T.W., Ingram, D.S. & Rawlinson, C.J. (1984). Heterothallism in *Pyrenopeziza brassicae*, cause of light leaf spot of brassicas. – Transactions of the British Mycological Society 82: 477-483.

Majer, D., Mithen, R., Lewis, B.G., Vos, P. & Oliver, R.P. (1996). The use of AFLP fingerprinting for the detection of genetic variation in fungi. – Mycological Research 100: 1107-1111.

Purwantara, A., Barrins, J.M., Cozijnsen, A.N., Ades, P.K. & Howlett, B.J. (2000). Genetic diversity of isolates of the *Leptosphaeria maculans* complex in Australia, Europe and North America using amplified fragment length polymorphism analysis. – Mycological Research 104: 772-781.

Simons, A.J. & Skidmore, D.I. (1988). Race-specific resistance to light leaf spot in *Brassica oleracea*. – Transactions of the British Mycological Society 90:431-435.

Light leaf spot

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 231-236

Detection of *Pyrenopeziza brassicae* (light leaf spot) infection of winter oilseed rape

Zbigniew R. Karolewski[1,2], Bruce D.L. Fitt[1], Akinwunmi O. Latunde-Dada[1], Neal Evans[1] and Simon J. Foster[1]

[1] *Rothamsted Research, Harpenden, AL5 2JQ, UK*
[2] *University of Agriculture, Dabrowskiego 159, 60-594 Poznan, Poland*

Abstract: After initial infection of winter oilseed rape leaves in autumn by air-borne ascospores, *Pyrenopeziza brassicae* has a long symptomless phase before the first visible necrotic lesions appear, in January/February in the UK or March/April in Poland. Assessment of symptomless *P. brassicae* infection visually is not reliable before necrotic lesions appear, unless plants are first incubated for several days at high humidity, in polyethylene bags, to encourage *P. brassicae* sporulation. Visual methods for assessment of light leaf spot in winter oilseed rape were compared with PCR for detection of *P. brassicae* infection. PCR diagnosis on leaves sampled from field experiments was more sensitive than visual assessments, even after incubation, with symptomless infection of leaves detected 2 months earlier by PCR than by incubation in polyethylene bags.

Key words: *Brassica napus,* disease assessment, PCR diagnostics, symptomless infection

Introduction

During epidemics of light leaf spot in winter oilseed rape in Europe, *Pyrenopeziza brassicae* has a long symptomless phase after initial infection of leaves in autumn by air-borne ascospores. The first visible lesions appear in January/February (Figueroa *et al*., 1995; Fitt *et al*., 1998; Gilles *et al*., 2000). Su *et al.* (1998) suggested that it is possible to predict potential yield loss caused by light leaf spot from assessments made at GS 3,3 (flower buds visible) (Sylvester-Bradley & Makepeace, 1985), when disease incidence (% plants affected) is assessed after incubation of plants in polyethylene bags. Another potential method for assessing light leaf spot is the detection of symptomless infection by use of PCR. *P. brassicae* DNA was detected by PCR assay in oilseed rape leaves (cv. Bristol), using primers Pb1 and Pb2. These detected *P. brassicae* DNA in leaves inoculated with conidia 13 days after inoculation in a controlled environment (Foster *et al*., 2002). Using a more time consuming nested PCR protocol, detection was possible at 6 days after inoculation (Foster *et al*., 2002). There is a need to test PCR methods for detection of symptomless *P. brassicae* infection of winter oilseed rape leaves sampled in the field. The paper compares PCR detection with visual assessment of light leaf spot done directly in the field (*in situ*) and in the laboratory after incubation in polyethylene bags.

Material and methods

Controlled environment experiments

To compare sensitivity of primer pairs Pb1 & Pb2 and PbITSF & PbITSR for detection of *P. brassicae* DNA in oilseed rape leaves, thirty oilseed rape plants (cv. Darmor) grown in compost in plastic pots (diameter 9 cm) were inoculated at GS 1,6-1,7 by spraying leaves 5

and 6 with a suspension of conidia (0.5 x 10^6 conidia mL^{-1}) until droplets ran off the leaf surfaces. Plants were maintained in controlled environment cabinets for 14 days after inoculation. Every day, one leaf (leaf 5 or leaf 6) was sampled at random and stored at −20 ^oC. DNA was extracted from leaves collected using a protocol modified from that of Lee and Taylor (1990). Leaves (c. 3g) were ground separately in liquid nitrogen using a pestle and mortar and approximately 300 mg of ground leaf tissue was suspended in 600 µL of lysis buffer (50 mM Tris HCL ph 7.4 at 37 ^oC, 50 mM EDTA, 3% sodium dodecyl sulphate, 1% beta-mercaptoethanol) and incubated at 65 ^oC for 1h. Samples were extracted twice with an equal volume of phenol/chloroform (1:1 v/v). DNA was precipitated from the aqueous phase by addition of 40 µL of 6 M ammonium acetate and 600 µL isopropanol at −20 ^oC. After centrifugation, DNA pellets were washed with 1.0 mL 70% ethanol, air dried for 25 min and resuspended in 100 µL TE buffer (10 mM Tris HCL pH 7.5 at 25 ^oC, 1 mM EDTA). DNA concentration was determined spectrophotometrically at 260 nm. For PCR reactions, DNA samples were adjusted to 100 ng µL1. In reactions primers Pb1 (5`-CAA CAT TGC CTG GTA TTG AGA AAC-3`) and Pb2 (5`-ATC TGA TAC GCC TAC ACCGTC C-3`) (Foster *et al*., 2002) were compared with a new pair PbITSF (5'-TTGAACCTCTCGAAGAAGTT CAGTCT-3') and PbITSR (5'-AGATTTGGGGGTTGTTGGCTAA-3'). Cycling parameters and visualisation of PCR products on a u.v. transilluminator described in Foster *et al*. (2002) were the same for both pairs of primers.

In the next experiment symptomless *P. brassicae* infection was detected using PCR primers PbITSF and PbITSR on leaves of oilseed rape Darmor (UK resistance rating 4) and Eurol (7). 30 plants of each oilseed rape cultivar were inoculated at GS 1,6-1,7. Inoculation with *P. brassicae* conidia and extraction of DNA were done as described previously. Plants were maintained in controlled environment cabinets for 14 days after inoculation. Every day, one leaf (leaf 5 or leaf 6) from one plant of each cultivar was removed at random and stored at −20 ^oC. For PCR reactions, DNA samples from cv. Darmor were diluted 10, 100 or 1000 times from the original concentrations. Samples from cv. Eurol were adjusted to 100, 250, 500 or 1000 ng μL^{-1}. Extraction of DNA and PCR reactions were done using methods described for the first experiment.

Field experiment

The winter oilseed rape experiment was done in the 2003/2004 growing season on the Rothamsted farm. Seeds of six cultivars were sown on 22 August 2003 (80 seeds/m^2) in plots (20m x 3m) arranged in three replicate blocks. Three of the cultivars were bred for Polish climatic conditions (Bosman, Kana, Marita) and three were from the UK recommended list, with different resistance ratings (0-9 scale: 0 = susceptible, 9 = resistant, www.hgca.com) (Apex (5), Canberra (8), Recital (6)). Between plots with test cultivars, plots of the same size were sown with the susceptible cultivar Shannon (3). Assessments of light leaf spot as a percentage of leaves affected were done every 4 weeks. Both laboratory and *in situ* assessments were done. For the laboratory assessments, 10 plants (with roots) were sampled from each of the 18 plots and incubated for 5 days at 8^oC in polyethylene bags (Fitt *et al*., 1998). For PCR detection of symptomless infection 10 leaves from one plot of each cultivar were sampled regularly and PCR diagnosis was done using the primer pair PbITSF/PbITSR. The oldest leaves were taken from 10 plants at random each month from 9 December 2003 until 15 March 2004 (when light leaf spot symptoms were first visible). Directly after sampling, leaves were washed in water, dried for 30 min at 20^{o}C and frozen at -20^{o}C. DNA was extracted from leaves collected, using a protocol modified from that of Lee & Taylor (1990). For PCR reactions, DNA samples were adjusted to concentrations of 100, 200, 500 or 1000 ng μL^{-1} DNA.

Results and discussion

Controlled environment experiments

P. brassicae DNA was detected in PCR reactions from all samples of cv. Darmor adjusted to 100 ng μL^{-1} using the ITS set of primers (Fig. 1), whilst the Pb1 and Pb2 primers did not amplify samples collected on the third and eighth days after inoculation. These results suggest that the new pair of primers ITSF/ITSR can be used for detection of symptomless *P. brassicae* infection of winter oilseed rape leaves in crops and field experiments.

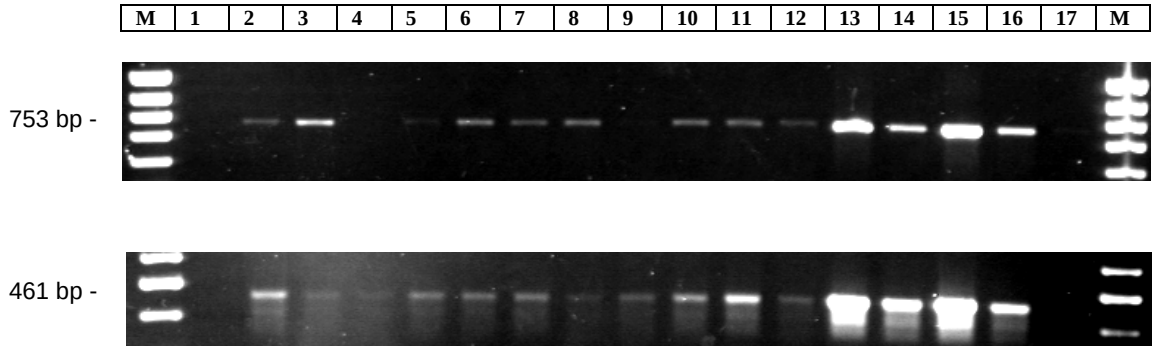


Figure 1. PCR amplification of *P. brassicae* DNA extracted from leaves of oilseed rape cv. Darmor inoculated with *P. brassicae* conidia, using primers Pb1 and Pb2 (a) or PbITSF and PbITSR (b). DNA concentration was adjusted to 100 ng μL^{-1}. M, 100 bp DNA ladder (MBI Fermentas, Lithuania); lane 1, DNA from uninoculated leaf; lanes 2-15, DNA from inoculated leaves collected 1-14 days after inoculation; lane 16, 1 ng *P. brassicae* isolate NH10 genomic DNA; lane 17, negative (water) control.

P. brassicae DNA in the pool of undiluted DNA extracted from infected leaf samples (cv. Darmor) collected 9, 10, 11, 12, 13 and 14 days after inoculation was amplified in PCR reactions using ITS primers (Table 1a). Furthermore, the PCR products from all samples diluted 10x were visible, except for samples from 3 days after inoculation. Fungal DNA diluted 100x was detected in samples taken 1, 2, 12, 14 days after inoculation. At 14 days after inoculation, the PCR product was visible when the DNA sample was diluted 1000 fold. Using the ITS set of primers, *P. brassicae* DNA was detected in all samples from oilseed rape leaves (cv. Eurol) adjusted to 100 ng μL^{-1} except samples collected 3 and 6 days after inoculation (Table 1b). It was also detected in PCR reactions from all samples adjusted to 1000 ng μL^{-1} except the sample collected 12 days after inoculation. DNA adjusted to 500 ng μL^{-1} gave successful amplification in all samples except 6 days after inoculation, whilst adjustment to 250 ng μL^{-1} gave no *P. brassicae* products in samples harvested 3, 6, 9 and 10 days after inoculation.

Field experiment

In the 2003/2004 winter oilseed rape experiment, symptoms of light leaf were not observed during assessments done between December and March when plots were assessed in the field without incubation (Table 2.). Sporulation of *P. brassicae* appeared in March on leaves of all cultivars except for cv. Kana, although the percentage of leaves affected was not higher than 9% (cv. Kana). *P. brassicae* DNA was detected using PCR in leaves of cvs. Kana and Recital in January, whilst in leaves cvs. Bosman, Canberra and Marita in February. In March the highest percentage of infected leaves using PCR was found for cv. Canberra (50%).

Table 1. *P. brassicae* infection of individual leaves (5 or 6) of oilseed rape cv. Darmor (a) or cv. Eurol (b), inoculated with conidia (0.5×10^6 mL^{-1}) at GS 1,6-1,7, assessed by PCR[1] on inoculated leaves (experiment 4).

A) cv.Damor [2]	**Days after inoculation**													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
DNA dilution	**DNA concentration [ng mL^{-1}]**													
	2290	2280	1980	1840	1750	1740	1690	1650	1520	1270	1110	1020	1010	640
Undiluted	I	I	–	–	–	+	–	+	+	+	–	+	+	+
10 fold	+	+	–	+	+	+	+	+	+	+	+	+	+	+
100 fold	+	I	–	–	+	–	–	–	+	–	–	–	–	+
1000 fold	I	I	–	–	–	–	–	–	–	–	–	–	–	+

B) cv. Eurol [2]

DNA conc. ng mL^{-1}	**Days after inoculation**													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
100	+	+	–	+	+	–	+	+	+	+	+	+	+	+
250	+	+	–	+	+	–	+	+	–	–	+	+	+	+
500	+	+	+	+	+	–	+	+	+	+	+	+	+	+
1000	+	+	+	+	+	+	+	+	+	+	+	–	+	+

[1] PCR detection of *P. brassicae* DNA done using primers PbITSF and PbITSR on individual leaves (5 or 6) sampled at random.
[2] DNA extracted from oilseed rape leaves (cv. Darmor) was diluted 10, 100 or 1000 times; concentration of DNA extracted from oilseed rape leaves (cv. Eurol) was set to 100, 250, 500 or 100 ng mL^{-1}

Table 2. Comparison of methods for visual or PCR assessment of light leaf spot (*P. brassicae*) on winter oilseed rape leaves sampled from a field experiment at Rothamsted in 2003/2004

Cultivar	Sampling date	% leaves infected by *P. brassicae*		
		Visual *in situ* (without incubation)	Visual (after incubation)	PCR (without incubation)
Apex	7 Dec	0	0	0
	9 Jan	0	0	0
	12 Feb	0	0	0
	12 Mar	0	9	40
Bosman	7 Dec	0	0	0
	9 Jan	0	0	0
	12 Feb	0	0	20
	12 Mar	0	5	30
Canberra	7 Dec	0	0	0
	9 Jan	0	0	0
	12 Feb	0	0	20
	12 Mar	0	2	50
Kana	7 Dec	0	0	0
	9 Jan	0	0	10
	12 Feb	0	0	0
	12 Mar	0	0	40
Marita	7 Dec	0	0	0
	9 Jan	0	0	0
	12 Feb	0	0	10
	12 Mar	0	2	30

Conclusions

PCR detection of *P. brassicae* DNA extracted from oilseed rape leaves inoculated with conidia in controlled environment showed that the pair of primers PbITSF and PbITSR can be used for such tests because this pair was more sensitive than Pb1 and Pb2. PCR detection of *P. brassicae* from oilseed rape leaves inoculated in controlled environment experiment indicates that, for cultivars with a low level of resistance, the extracted DNA should be diluted 10 times to obtain a concentration of about 100-200 ng mL^{-1}. For more resistant cultivars the concentration should be higher - 500 ng mL^{-1}). This is due to the higher percentage of fungal DNA in total DNA extracted from leaves sampled from susceptible cultivars. In this case, PCR reaction is inhibited by too high a concentration of fungal DNA. Results from the field experiment suggest that methods involving incubation of samples were more sensitive than assessments *in situ* in field plots. However PCR diagnosis on leaves sampled from field experiments was the most sensitive, even after incubation, with infection detected on leaves 2 months earlier. This suggests that visual assessment of light leaf spot

should be supplemented by sampling and incubation of plants (at 8-10ºC for 5 days) or by PCR diagnostics.

Acknowledgements

The research was supported by the European Commission - INCO A1 programme "States in the phase of pre-accession to the European Union" (contract ICA1-CT-2002-70005), the UK Biotechnology and Biological Sciences Research Council and Department for Environment, Food and Rural Affairs, the Home-Grown Cereals Authority and GIE ProColza.

References

Figueroa, L., Fitt, B.D.L., Welham, S.J., Shaw, M.W. & McCartney, H.A. 1995. Early development of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in relation to temperature and leaf wetness. – Plant Pathology 44: 641-654.

Fitt, B.D.L., Doughty, K.J., Gladders, P., Steed, J.M. & Sutherland, K.G. 1998. Diagnosis of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. – Annals of Applied Biology 133: 155-166.

Foster, S.J., Ashby, A.M. & Fitt, B.D.L. 2002. Improved PCR-based assays for pre-symptomatic diagnosis of light leaf spot and determination of mating type of *Pyrenopeziza brassicae* on winter oilseed rape. – European Journal of Plant Pathology 108: 379-383.

Gilles, T., Evans, N., Fitt, B.D.L. & Jeger, M.J., 2000. Epidemiology in relation to methods for forecasting light leaf spot (*Pyrenopeziza brassicae*) severity on winter oilseed rape (Brassicae napus) in the UK. – European Journal of Plant Pathology 106: 593-600.

Lee, S.B. & Taylor, J.W. 1990. Isolation of DNA from fungal mycelia and single spores. – In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.). PCR Protocols. A guide to Methods and Applications. San Diego, CA, USA, Academic Press: 282-287.

Su H., Fitt B.D.L., Welham S.J., Sansford C.E. & Sutherland K.G., 1998. Effects of light leaf spot (*Pyrenopeziza brassicae*) on yield of winter oilseed rape (*Brassica napus*). – Annals of Applied Biology 132: 371-386.

Sylvester-Bradley R. & Makepeace R.J., 1985. Revision of a code for stages of development in oilseed rape (*Brassica napus*). – Aspects of Applied Biology 10: 395-400.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 237-243

Development of light leaf spot and phoma stem canker on a range of current and historical oilseed rape cultivars at Rothamsted in 2003/04 and 2004/05 growing seasons.

Katherine Downes, Neal Evans, Elizabeth Pirie and Bruce D.L. Fitt
Rothamsted Research, Harpenden, AL5 2JQ, UK

Abstract: Field trials in successive growing seasons (2003/04 and 2004/05) were monitored for light leaf spot (*Pyrenopeziza brassicae*), phoma leaf spot and phoma stem canker (*Leptosphaeria maculans*) infection. Each experiment included 42 current, historical or exotic cultivars/breeding lines. Weekly assessments examined leaf, stem and pod disease as % plants affected and % area affected. Significant differences were found between resistant and susceptible cultivars, suggesting resistance to be under discrete genetic control. Many of the cultivars that were susceptible or resistant in the 2003/04 season gave similar responses in 2004/05. However, material gave a range of responses to the two pathogens; for example, cv. Aviso was very resistant to *L. maculans* in both seasons whilst Bronowski was the most susceptible cultivar in both seasons. For *Pyrenopeziza brassicae*, cv. Tapidor was consistently susceptible whilst the line PR45W05 was consistently resistant, when % leaf area affected was assessed. The results are discussed in relation to current knowledge of the reported oilseed rape resistance gene mediated response to *L. maculans* and the suggested resistance mechanism of oilseed rape to *P. brassicae*.

Keywords: *Brassica napus*, *Cylindrosporium concentricum*, disease assessment, *Leptosphaeria maculans*, *Phoma lingam*, *Pyrenopeziza brassicae*, resistance.

Introduction

Pyrenopeziza brassicae and *Leptosphaeria maculans* are two of the most important pathogens of oilseed rape, causing considerable yield losses. Light leaf spot (*P. brassicae*, anamorph *Cylindrosporium concentricum*) is a polycyclic disease that infects oilseed rape leaves, stems, flowers and pods during the course of the growing season (McCartney & Lacey, 1990; Gilles *et al*., 2000). Phoma stem canker (blackleg), caused by *L. maculans* (anamorph *Phoma lingam*), is a monocyclic disease. *L. maculans* infects leaves in the autumn, grows systemically to the stem and causes damaging stem cankers in spring/summer (West *et al*., 2002). In the UK both diseases have caused losses in excess of £20 million per season since 1993, despite the use of fungicides (Defra survey results; http://cropmonitor.co.uk/).

The mechanism of resistance to *L. maculans* operating in the leaf has been described as a gene-for-gene relationship, where the resistance or susceptibility of a cultivar depends on the presence of a major gene for resistance (*R*) in the host and a corresponding "effector" (*AVR*) gene in the pathogen. Specific recognition leads to the rapid onset of plant defence responses to fully protect the plant from the pathogen. There are currently nine resistance genes described (Delourme *et al*., 2004), which correspond to nine effector genes in *L. maculans*. The understanding of the resistance mechanism in the *Brassica napus – P. brassicae* interaction is less well defined. In this experiment, a range of current and historical cultivars and novel and exotic breeding material were used to investigate resistance responses to field populations of *L. maculans* and *P. brassicae* at Rothamsted.

Materials and methods

In both the 2003/04 and 2004/05 seasons, three replicate blocks of 42 current or historical cultivars, breeding lines or exotic material were arranged at random in lines (size 2m x 0.25m). The 2003/04 and 2004/05 experiments were sown on 11/12 September 2003 and 03 September 2004, respectively. Guard rows of the susceptible cultivar Shannon were sown on either side of lines to decrease edge effects and inter-plot interference and encourage light leaf spot development. To increase the severity of the subsequent light leaf spot epidemic, the experiments were inoculated indirectly with upper stem and pod debris from the oilseed rape crop harvested the previous season. Upper stem and pod debris (one bale for the whole experimental area) was placed around the experimental area and along paths and tractor wheelings. Application of debris directly to the lines would have produced unusually early, severe disease initiated by rain-splashed conidia or direct contact. Inoculation was done a few weeks after seedling emergence.

Plots were assessed weekly once infection was first observed. Four disease assessments were done per line; number of plants out of 10 with *P. brassicae* infection and % leaf area affected with *P. brassicae*, number of plants out of 10 with *L. maculans* infection and % leaf area affected with phoma leaf spot. In addition, growth stage of each line was evaluated using the scale derived by Sylvester-Bradley & Makepeace (1985). Stem and pod disease were assessed weekly from first signs of disease until final harvest. Prior to harvest 10 plants per line were sampled and bagged for final assessment. The assessment recorded not only the % stems and % pods affected but also height and phoma stem canker severity. Canker severity was assessed by cutting through the base of the stem at the root collar and scoring any canker on a 0-4 scale (0 (no canker) to 4 (stem completely girdled and discoloured)).

Results and discussion

Field experiments in 2003/04 and 2004/05

During both seasons, the material showed a range of responses to the two pathogens, with some cultivars being susceptible and others resistant. Figure 1 shows the response of material to *P. brassicae* as shown by % stem area with light leaf spot at harvest for the 2003/04 (Fig. 1a) and 2004/05 seasons (Fig. 1b). Some material gave a similar response in both seasons; for example PR4SW05, Escort and Mohican were consistently resistant whilst Tapidor, Aviso and Bronowski were consistently susceptible. However, some material gave different results depending on the season; for example Surpass, which gave a resistant response in the 2003/04 season gave a susceptible response in the 2004/05 season (Fig.1).

A similar response was observed for % pods affected with light leaf spot (Fig. 2). Cv. Tapidor was consistently susceptible with severe pod disease whilst cultivars such as Twister and Mohican gave a consistently resistant response. However, there were some cultivars which did not give a consistent response; for example Surpass was resistant in the 2003/04 season (Fig. 2a) and highly susceptible in the 2004/05 season (Fig. 2b).

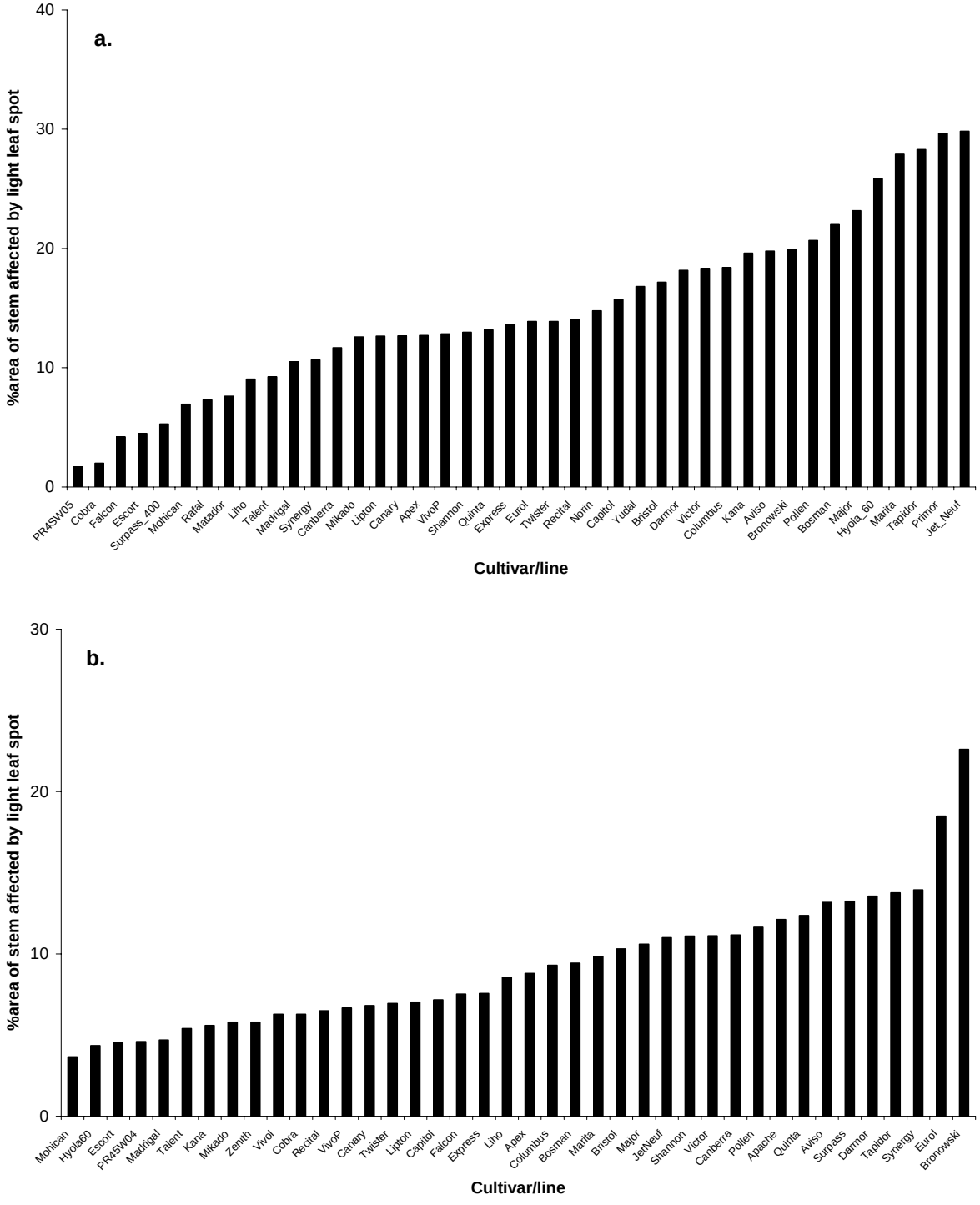


Figure 1. Percentage of oilseed rape stem area affected with light leaf spot (*P. brassicae*) for a range of current or historic oilseed rape cultivars or breeding material at Rothamsted during the 2003/04 (a. S.E.D. = 5.4, d.f. = 70) and 2004/05 (b. S.E.D. = 6.7, d.f. = 67) growing seasons.

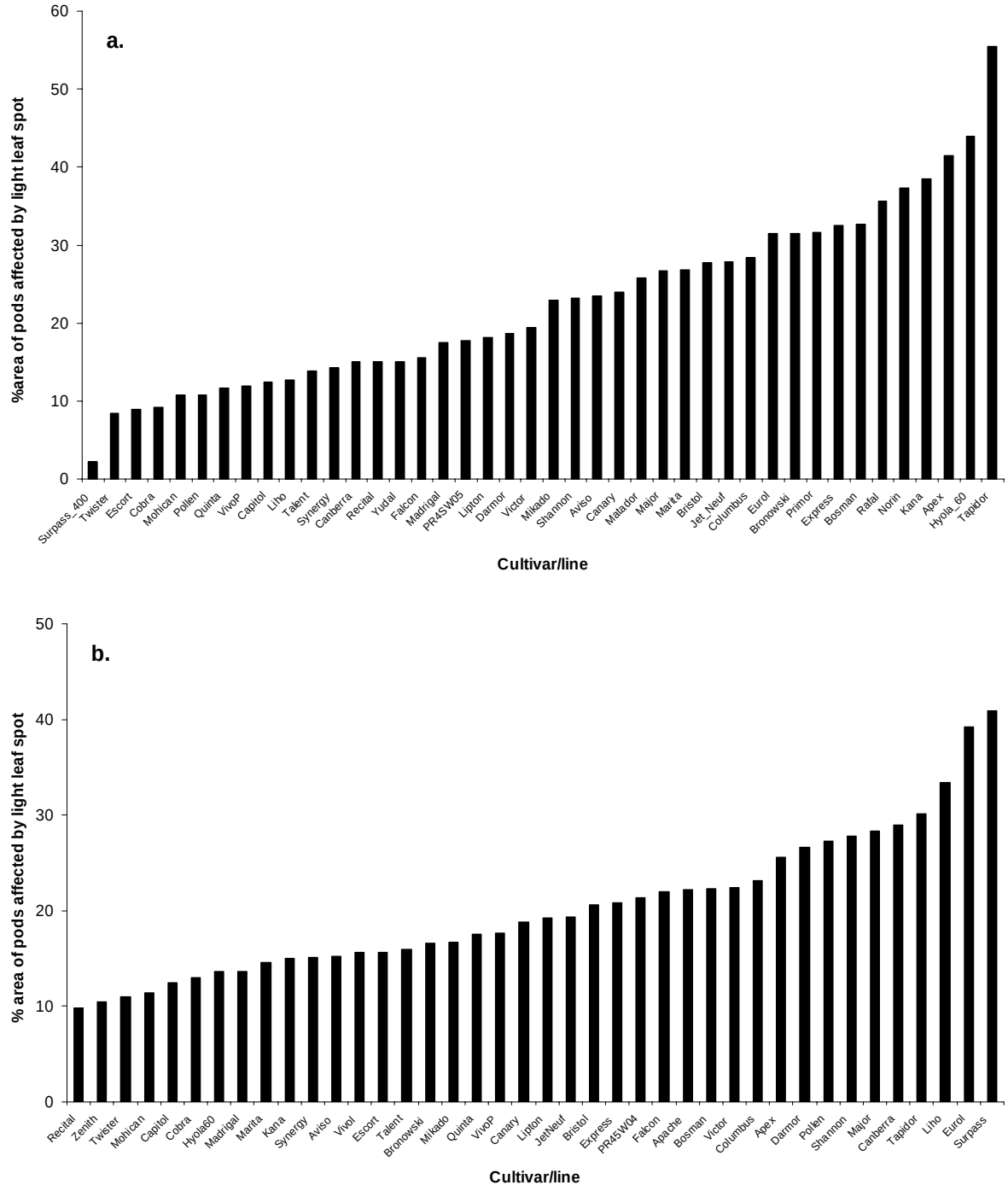


Figure 2. Percentage area of oilseed rape pods affected with light leaf spot (*P. brassicae*) for a range of current or historical oilseed rape cultivars or breeding material at Rothamsted during the 2003/04 (a. S.E.D. = 10.1, d.f. = 69) or 2004/05 (b. S.E.D. = 6.67, d.f. = 67) growing seasons.

With respect to phoma stem canker, in both seasons, phoma leaf spotting was greatest in early December then decreased rapidly when first leaves senesced (data not shown). At harvest, material showed a range of responses for stem canker severity (scored on a 0-4 scale, no disease to 100% stem girdled).

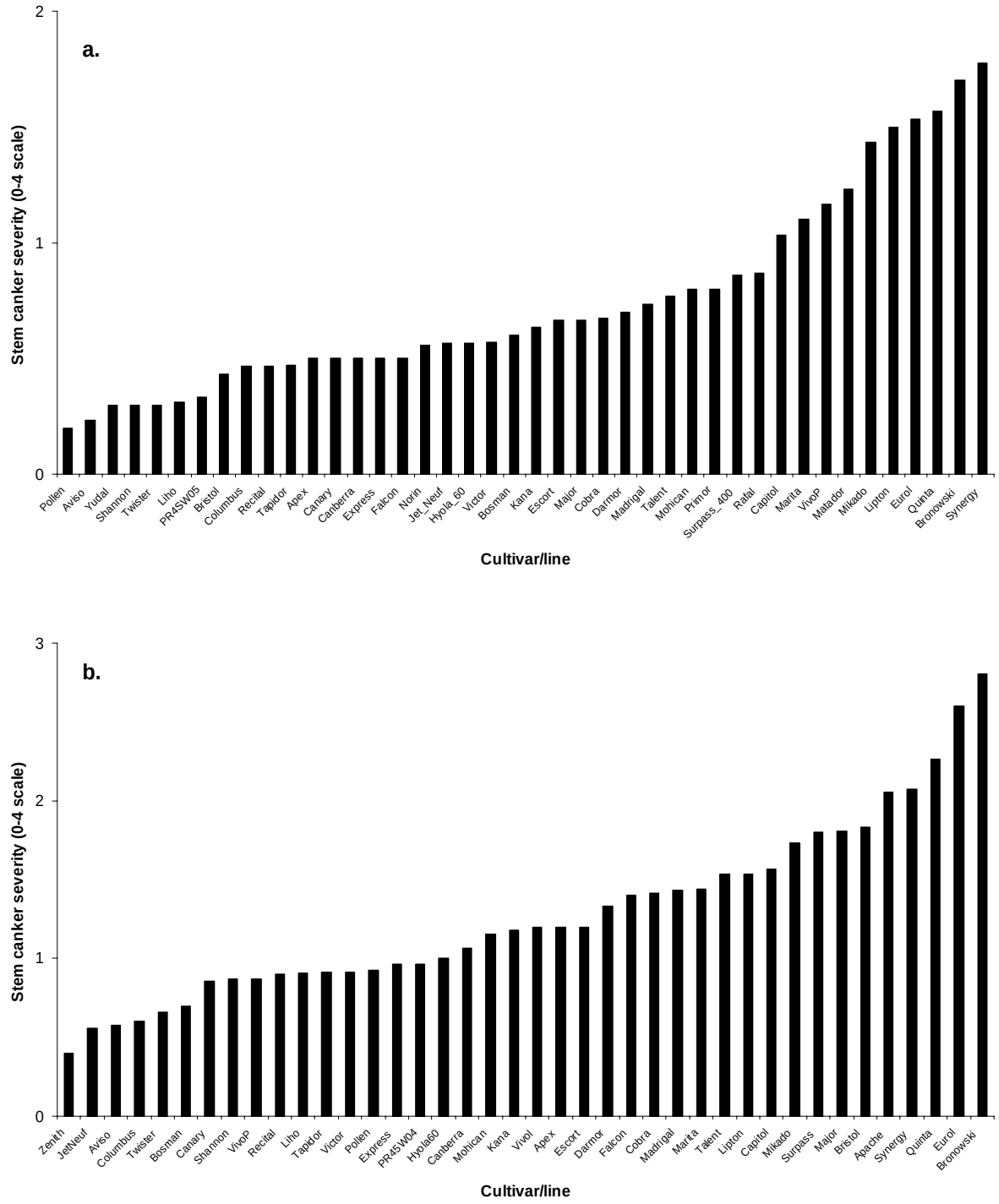


Figure 3. Stem canker severity score for oilseed rape plants affected with phoma stem canker (*L. maculans*) for a range of current or historical oilseed rape cultivars or breeding material at Rothamsted, assessed pre-harvest at the end of the 2003/04 (a. S.E.D. = 0.39, d.f. = 75) or 2004/05 (b. S.E.D. = 0.42, d.f. = 67) growing seasons.

There was a range of responses for the different lines with respect to stem canker severity rating. A number of cultivars, such as Recital and Shannon, were consistently resistant in both the 2003/04 (Fig. 3a) and 2004/05 seasons (Fig. 3b), whilst cultivars such as Brownowski, Synergy and Quinta were consistently susceptible and developed severe cankers and had a

high canker severity score in both seasons. Response to *L. maculans* was much less variable than the response to *P. brassicae*. Correlations between the 2003/04 season results and the 2004/05 season results for stem canker severity produced a very high coefficient (0.78) and an R^2 value of 0.61 (P<0.001).

The correlation between the results for the 2003/04 and the 2004/05 seasons for stem canker severity suggests that the resistance of a cultivar to stem canker is under discrete genetic control and that environment has less effect on the severity of stem cankers then of light leaf spot. The response of material was uniform, considering seasonal differences in crop growth and epidemic progress. The most probable explanation for this good correlation was the presence of the *Rlm* genes in most current and historical oilseed rape cultivars that correspond to effector genes in the *L. maculans* pathogen (Rouxel & Balesdent, 2005). The similarities in the results from the two seasons also suggest that the pathogen population did not change significantly from one season to the next. However, this is not surprising since the areas/numbers of plants grown in the experiments were very small and the successive experiments were done in fields that were separated by a distance of approximately 0.25 km.

In contrast, the response of material with respect to % stem area affected and % pod area affected by light leaf spot over the two seasons suggests more of an environmental effect. Rainfall during October 2004 was very high (146 mm) producing exceptionally favourable conditions for the initial development of light leaf spot as severity is weather dependent (Cheah & Hartill, 1985; Fitt *et al*., 1999; Evans *et al*., 2000). In the 2002/2003 season, rainfall in October was also high (127.2 mm) and light leaf spot was first detected in February (Karolewski *et al*., 2005). However in the 2004/2005 season, light leaf spot was observed in mid-December, indicating a severe early epidemic. In the 2003/2004 season, the epidemic was much later because there was less rain during autumn. Rainfall affects development of apothecia and subsequent production of ascospores and thus infection of plant leaves (Gilles *et al*., 2000).

There was discrimination between cultivars for both diseases. However, for light leaf spot, the use of a more natural inoculum would provide a better test of resistance response as inoculum concentration was high during the current study. This could be achieved by doing future experiments in an area where light leaf spot is the major oilseed rape disease, such as in Scotland (Sutherland *et al*., 1998).

Acknowledgements

We thank ProColza and the Home-Grown Cereals Authority for funding this work and Defra (UK Department for Environment Food and Rural Affairs) Sustainable Arable LINK for sponsorship. We also thank Douglas Arkell for help with collecting experimental data. Rothamsted Research receives funding from the UK Biotechnology and Biological Sciences Research Council.

References

Cheah, L.H. & Hartill, W.F.T. 1985. Disease cycle in New Zealand of light leaf-spot of Brassicas, induced by *Pyrenopeziza brassicae*. – New Zealand Journal of Agricultural Research 28: 567-573.

Delourme, R., Pilet-Nayel, M.L., Archipiano, M., Horvais, R., Tanguy, X., Rouxel, T., Brun, H., Renard, M., & Balesdent, M.H. 2004. A cluster of major specific resistance genes to *Leptosphaeria maculans* in *Brassica napus*. – Phytopathology 94: 578-583.

Fitt, B.D.L., Doughty, K.J., Gilles, T., Gladders, P., Steed, J.M., Su, H. & Sutherland, K.G. 1998. Methods for assessment of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. – Annals of Applied Biology 133: 329-341.

Fitt, B.D.L., Doughty, K.J., Gladders, P., Steed, J.M. & Sutherland, K.G. 1998. Diagnosis of light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape (*Brassica napus*) in the UK. – Annals of Applied Biology 133: 155-166.

Gilles, T., Evans, N., Fitt, B.D.L., & Jeger, M.J. 2000. Epidemiology in relation to methods for forecasting light leaf spot (*Pyrenopeziza brassicae*) severity on winter oilseed rape (*Brassica napus*) in the UK. – European Journal of Plant Pathology 106: 593-605.

McCartney, H.A., Lacey, M.E. & Rawlinson, C.J. 1986. Dispersal of *Pyrenopeziza brassicae* spores from an oil-seed rape crop. – Journal of Agricultural Science, Cambridge 107: 299-305.

Rouxel, T. & Balesdent, M.H. 2005. The stem canker (blackleg) fungus, *Leptosphaeria maculans,* enters the genomic age. – Molecular Plant Pathology 6: 225-241.

Sylvester-Bradley, R. & Makepeace, R.J. 1985. Revision of a code for stages of development in oilseed rape (*Brassica napus*). – Aspects of Applied Biology 10 (Field trials methods and data handling): 395-400.

West, J.S., Balesdent, M.H., Rouxel, T., Narcy, J.P., Huang, Y-J., Roux, J., Steed, J.M., Fitt, B.D.L. & Schmit, J. 2002 Colonisation of winter oilseed rape tissues by A/Tox^{+} and B/Tox° *Leptosphaeria maculans* (phoma stem canker) in France and England. – Plant Pathology 51: 311-321.

Blackleg, stem canker

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 247-254

Phomadidacte: A computer-aided training program for the severity assessment of phoma stem canker of oilseed rape

Jean-Noel Aubertot [1], Yassine Sohbi [1], Hortense Brun [2], Annette Penaud [3] and Forrest W. Nutter, Jr. [4]

[1] UMR d'Agronomie INRA/INA P-G, BP1, 78850 Thiverval-Grignon, France; [2] UMR BIO3P, Domaine de la Motte, 35653, Le Rheu Cedex, France; [3] CETIOM, BP4, 78850, Thiverval-Grignon, France; [4] Iowa State University, Dep. Plant Pathology, 351 Bessey Hall, Ames, IA 50011, USA

Abstract: Phoma stem canker (caused by *Leptosphaeria maculans*) is one of the main diseases that affect oilseed rape world-wide. The disease is usually characterised by the visual assessment of the severity of cankers created by the pathogen at the crown level of infected plants. In order to avoid arbitrary categories and to maintain standardisation of assessment keys, a rating scale based on the percentage severity estimates of cross-section cankered crowns has been recently proposed in France. This scale consists in 6 severity classes, defined as a function of the percentage of the discoloured cross-section: 1, healthy plant, no visible lesions; 2, 0-25%; 3, 25-50%; 4, 50-75%; 5, 75-100% of discoloured section; 6, section without any living tissue, plant lodged or broken at the crown level during sampling. However, like many other rating systems for the severity of diseases, assessor bias effects have been reported. The aim of this paper is first to give a description of Phomadidacte, a computer-aided training program to guide assessors on how to use this rating scale; and second, to report an evaluation of its efficacy in training assessors. The basic principle of the program consists of displaying pictures of cankered cross-sections of oilseed rape that are to be rated by the user. These pictures had been previously rated by a panel of eight experts from four different research or extension units (2 from INRA, 1 from CETIOM, and 1 from GEVES), and the mean of the severity classes assigned to each picture were taken as the "true" or correct severity class for each picture. At the end of a training session, the program will either suggest the assessor continues with training or will declare that there was good agreement between the experts and the user, based on two evaluation thresholds: the percentage of pictures correctly rated (75%) and the percentage of pictures for which the severity class given by the user and the expert differ by more than one severity class (5%). A graph summarising the differences between the user and the experts' grades is displayed and an ASCII file containing the data of the training session is created. The user can then browse all the pictures to compare the grades that the user gave with the experts' grades. Phomadidacte runs under Windows® and an on-line version is available. An experiment was conducted to test the efficacy of Phomadidacte in improving assessor accuracy. This experiment compared the grades given by two groups of ten assessors who have been evaluated either with or without using Phomadidacte with the grades assigned by a panel of three experts (INRA, CETIOM, GEVES) on actual diseased field samples. The group evaluated without Phomadidacte used one picture per severity grade and diagrams illustrating the diversity of symptoms that can be encountered. Plants were first graded by experts who chose twenty plants for each severity grade. The plants were ordered randomly from the field and then independently rated by all assessors. The distribution of errors (experts minus the assessors' grade) was significantly different between the two groups of assessors (Kolmogorov-Smirnov test, $P<10^{-4}$). The assessments for the group that had not received Phomadidacte training was in agreement with the experts in 62% of the cases, whereas the group trained with Phomadidacte achieved 69% agreement. Three assessors trained using Phomadidacte succeeded in having a percentage of agreement with experts greater than 75%, whereas none of the non-Phomadidacte group assessors succeeded in achieving this threshold. No assessor, within the two groups, had more than 4.2% of the cases with a difference with the experts that was greater than one severity class, which indicates that the scale is quite easy to use. The generic method described in this paper could be successfully applied to other pathosystems that are difficult to visually characterise.

Key words: Disease severity assessment, phytopathometry, *Leptosphaeria maculans*, blackleg, *Brassica napus*.

Introduction

The assessment of severity due to pest injuries (pathogens, weeds and animal pests) is critical for agriculture. For diseases, there are several reasons for assessing symptoms: to measure the efficacy of different types of control measures (*e.g.* cultivar resistance, chemical, biological, physical or cultural control); to determine damage functions, *i.e.* relationships between injuries and yield losses (that can be used to establish economic thresholds for pesticide applications); to perform regional agronomic diagnoses of commercial fields, such as those defined by Doré et al. (1997); to carry out epidemiological studies on pathogens; to analyse host-pathogen interactions. Several studies have been carried out on direct and indirect methods for disease assessment, and related sampling methods for different crops (Cooke, 1998). In the case of phoma stem canker (*Leptosphaeria maculans)*, one of the most severe diseases of oilseed rape world-wide (West *et al.*, 2001), several studies have been conducted to improve the characterisation of disease injury (Gilligan 1980; Pierre and Regnault, 1982; Rimmer and Van den Berg, 1992; Van den Berg *et al.*, 1993; Rempel and Hall, 1996; Aubertot *et al.*, 2004). However, unlike other pathosystems for which computer-aided training programs have been developed – *e.g.* DisTrain (Tomerlin and Howell, 1988); Disease.Pro (Nutter and Schultz, 1995); WinCombro (Canteri and Giglioti, 1998) – little efforts have been made to develop methods to help train assessors to assess the severity of phoma stem canker.

The basic principle of these computer-aided training programs consists of: i) displaying computer generated images of infected leaves whose visible diseased areas are known precisely; ii) letting the user rate these images; iii) comparing the user's rating with the computer generated reference. After each evaluation, these computer-aided training programs can be evaluated to determine if they improve the accuracy of assessors in estimating the percentage disease severity on a sampling unit (leaf). However, the pathosystems considered in these types of computer-aided training programs are foliar-spotting (*e.g.*, leaf rust, powdery mildew, septoria, scald, spot blotch, net blotch, leaf rust, stem rust on wheat; rust, early leaf spot, late leaf spot on peanut). In these cases, the computer generated references consist of symptoms represented with one or two colours positioned on monochromatic leaves (green) or pictures of leaves. In the case of phoma stem canker, the symptoms to assess are discoloured tissues of stem cross-sections that show a wide range of shades, so it is very difficult to generate reference computer images. In addition, field observations sometime present perturbing elements on the observed cross-sections: larva injuries, small soil aggregates, *etc*. The objective of this paper was i) to present a method to overcome these difficulties; ii) to describe Phomadidacte, a computer computer-aided training program for the severity assessment of phoma stem canker of oilseed rape; and iii) to present an evaluation of the efficacy of Phomadidacte to train assessors.

Materials and methods

Construction of visual references without computer generated images

In order to take into account the complexity of symptomatic cross sections to assess, one can consider using pictures to represent various injury classes to develop clear categories for reference. One way of producing such references categories would be to perform an

automated image analysis of pictures of symptoms, to assess, for instance, the proportion of diseased tissues. However, practice shows that such an approach is inappropriate because of the difficulty to define proper grey-level thresholds to distinguish diseased areas (pixels) from healthy tissues. These thresholds can vary from picture to picture, or even within a given picture. This is why we proposed to develop a set of disease reference images using the expertise of experienced assessors. The basic principal of the method consists of independent experts rating a set of pictures representing a wide range of disease severities, according to a given rating scale. If the majority of experts agree as to the grade of a specific picture, this picture is integrated into the reference image database. As with other computer-aided programs, this reference can subsequently be used to train assessors. One can remark that other authors have already accepted the rating provided by the developer of a rating scale to be the "true" or "actual" values for the disease severity of a sampling unit (O'Brien and van Bruggen, 1992).

Development of a reference for Phomadidacte

Phoma stem canker is usually characterised by the visual assessment of the severity of cankers created by the pathogen at the crown level of the plants. In order to avoid arbitrary categories and to maintain standardisation of assessment keys, a rating scale based on the percentage severity estimates of cross-sections of cankered crowns has been recently proposed (Aubertot *et al*., 2004). This scale consists of six severity classes defined as a function of the percentage of the discoloured cross-section: 1, healthy plant, no visible lesions; 2, 0-25% of discoloured cross-section; 3, 25-50% of discoloured cross-section; 4, 50-75% of discoloured cross-section; 5, 75-100% of discoloured section; 6, section without any living tissue, plant lodged or broken at the crown level during sampling (Aubertot *et al*., 2004). A set of pictures of more or less cankered cross-sections of oilseed rape stems was created in July 2002. These have been rated by a panel of eight experts from four different research or extension units (2 from INRA, 1 from CETIOM, and 1 from GEVES). This rating scale was used to build a reference image database of 120 pictures (20 pictures per severity class).

Structure of Phomadidacte

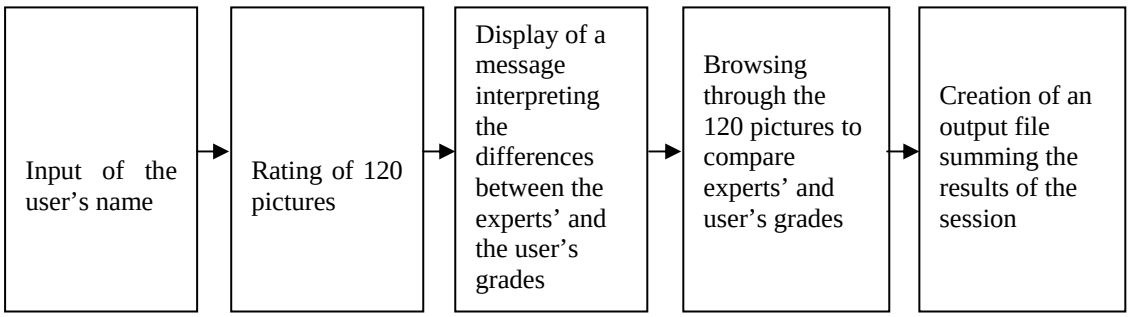


Figure 1. Process flow chart of Phomadidacte, a computer-aided training program for the severity assessment of phoma stem canker of oilseed rape caused by *Leptosphaeria maculans*.

The structure of Phomadidacte is presented Figure 1. After each assessor has specified their name, the user assigns each of the 120 references pictures (that appear in a random order) to one of the six severity classes (Figure 2). After all 120 disease images have been

graded, the distribution of assessor error (*i.e.*, the differences between the experts' grades and the corresponding assessor's grades) is displayed along with a short message that interprets the results. The message either congratulates the assessor for being in good agreement with the experts, or suggests the assessor (user) continue training before assessing Phoma stem canker severity in actual field experiments. The content of the message depends on two evaluation criteria: the proportion of pictures correctly graded and the proportion of pictures with a difference (from the expert's) greater than one severity class. In order to be congratulated, the percentage of pictures correctly rated has to reach at least 75% and the percentage of pictures for which the severity class given by the user and the experts differ by more than one severity grade has to be lower than 5%. These two evaluation thresholds were defined using the inter-variability of the experts: each expert would satisfactorily fulfil these two conditions when compared with the other 7 experts. A graph summarising the differences between the user and the experts' grades is displayed and an ASCII file containing the training session data is created for subsequent statistical analyses. The user can then browse all 120 pictures to compare the grades that they provided, versus the experts' grades (Figure 2). Phomadidacte operates under Windows® and an on-line version is available at: http://www-agronomie.grignon.inra.fr/fintranet.html. A login and a password will be supplied on request to the corresponding author (aubertot@grignon.inra.fr).

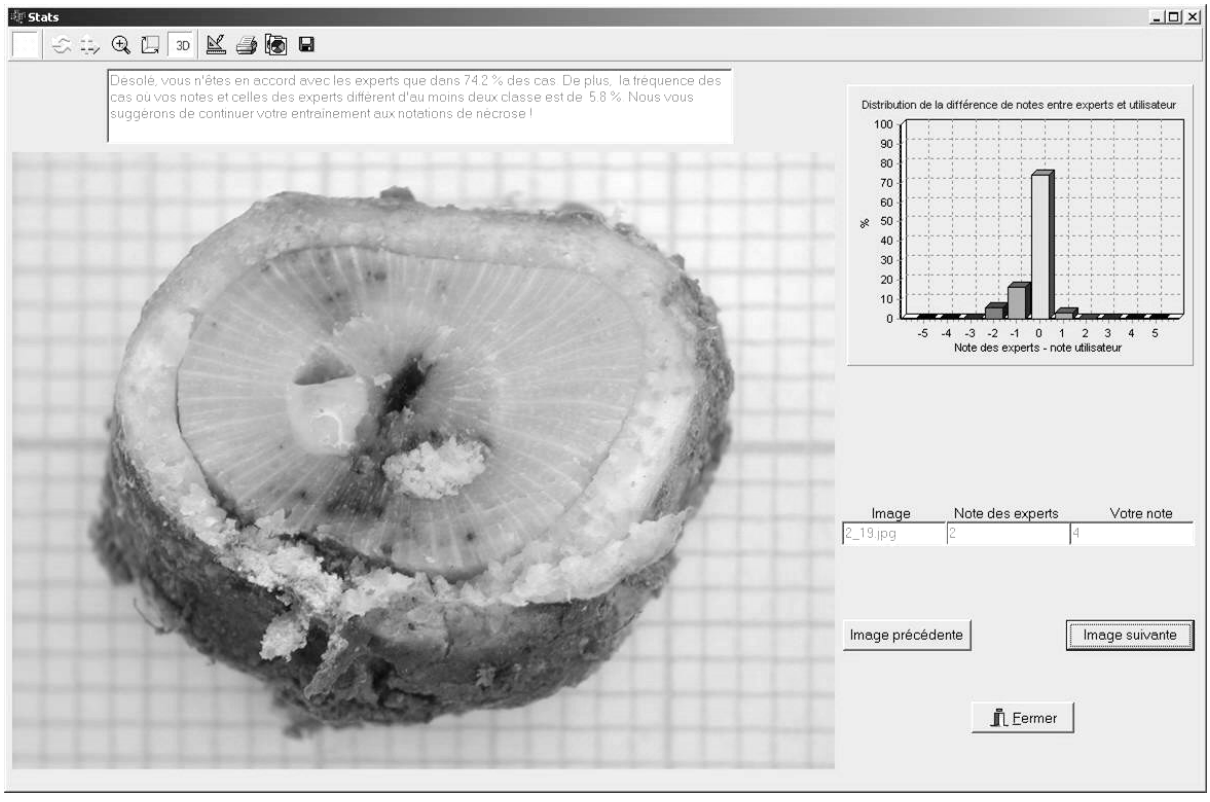


Figure 2. Screen image of Phomadidacte, a computer-aided training program for the severity assessment of phoma stem canker of oilseed rape, at the end of an evaluation and training session. A message indicates whether or not the user is in satisfactory agreement with the experts. A graph summarising the differences between the user and the experts' grades that were assigned for each of the 120 sampling units (pictures) is displayed. The user can then choose to browse all of the pictures to compare the grades that they provided versus those given by the experts' grades.

Evaluation of Phomadidacte

An experiment was done to compare the grades given by two groups of ten assessors who have been trained to assess Phoma stem canker disease of oilseed rape (caused by *Leptosphaeria maculans*) either with or without using Phomadidacte. The "true" or accurate disease severity class for each field sample was determined by a panel of three experts (one each from INRA, CETIOM, and GEVES). None of the twenty assessors had previously assessed the severity of Phoma stem canker on oilseed rape. The group trained without Phomadidacte used one picture per severity grade and diagrams illustrating the diversity of symptoms that might be encountered (Figure 3). Plants were first graded by experts who chose twenty plants for each severity grade. The plants were randomly reordered and then independently assessed by all the twenty assessors. We used SAS Release 6.12 for Windows (SAS Institute Inc., 1989) for statistical analyses. The distribution of errors (experts minus assessor's grades) was tested with the non-parametric Kolmogorov-Smirnov of the NPAR1WAY procedure.

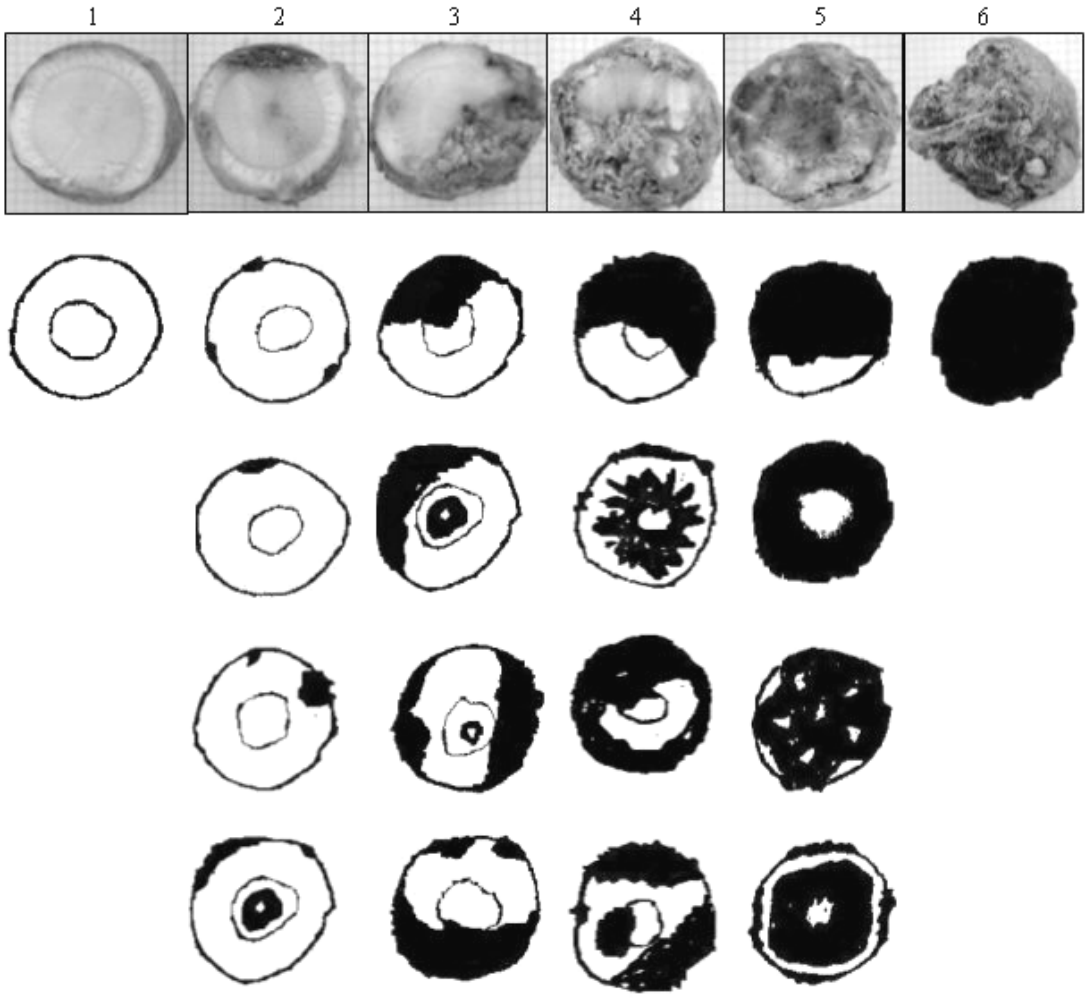


Figure 3. Images and sketches of cankered crown cross-sections of oilseed rape (Phoma stem canker, caused by *Leptosphaeria maculans*) classified into 6 severity classes: 1, healthy plant, no visible lesions; 2, 0-25% of discoloured cross-section; 3, 25-50%; 4, 50-75%; 5, 75-100% of discoloured cross-section; 6, section without any living tissue, plant lodged or broken at the crown level during sampling (Aubertot *et al.*, 2004). The graph paper used is graduated in millimeters.

Results and discussion

The distribution of errors (experts minus assessors' grades) was significantly different between the two groups of assessors (Figure 4; Kolmogorov-Smirnov test, $P<10^{-4}$). No difference greater than two severity grades were observed for both groups. No assessor (within the two groups) had more than 4.2% of the cases with a difference with the experts that was greater than one severity class. This indicates that the scale is quite easy to use, even when assessors are trained with diagrams instead of pictures.

The group trained without Phomadidacte was in agreement with the experts in 62% of the cases, whereas the group trained with Phomadidacte achieved 69% agreement. In addition to this increase of agreement between assessors and experts, Phomadidacte led to a symmetric distribution of errors (Figure 4), whereas the group trained without Phomadidacte overestimated the severity of symptoms as compared to the experts. Three assessors within the Phomadidacte-trained group succeeded in having a percentage of agreement with the experts greater than 75%, whereas none of the assessors in the control group succeeded in achieving this threshold.

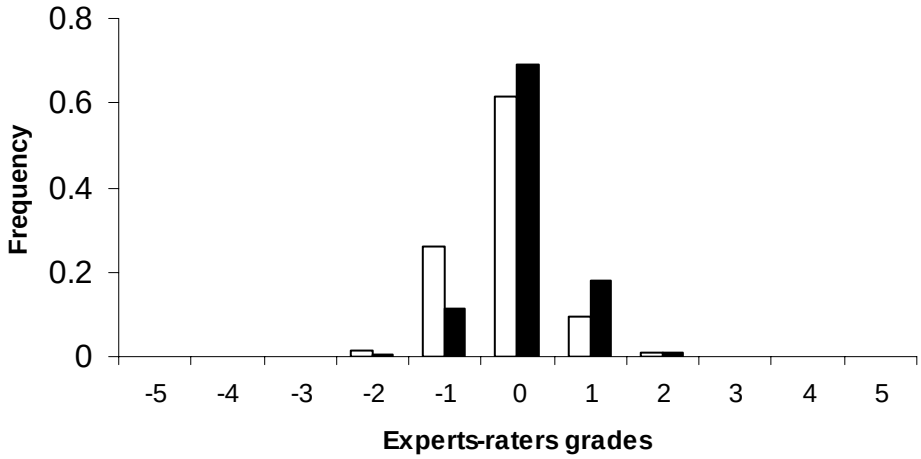


Figure 4. Distribution of errors (experts minus assessor's grades). □: represents the group of ten assessors trained with pictures and diagrams that are presented in Figure 3; ■: represents the group of ten assessors trained with Phomadidacte. Twenty oilseed rape cross-sections of crowns infected with *Leptosphaeria maculans* were used per severity grade were assessed by each group of ten assessors. The actual or "true" severity grades of each reference were estimated on real plants by three confirmed experts.

The main difficulty that is encountered when developing computer-aided training programs for disease severity assessment is the establishment of a "standard" to train or "calibrate" each assessor (Cooke, 1998). Image analysis could appear an objective way to quantify disease severity. However, since a threshold has to be used to distinguish healthy from diseased tissues, image analysis also suffers from a lack of objectiveness (Nutter *et al.*, 1993). The originality of Phomadidacte consists not only in using pictures of symptoms to train assessors, but also in using the expertise of a set of experts to develop a disease severity standard for each disease class. This approach should not be seen as an alternative to

computer-aided training programs that use generated diagrams, but as a complementary method where computer-generated images are not practical. Such programs are aimed to train assessors to assess the percentage of the diseased area on plant organs (leaves, generally) for contrasted objects (*e.g.* orange pustules of brown rust on green leaves of wheat), whereas for pathosystems such as phoma stem canker that express symptoms with a wide range of discolouration, and shapes within a disease severity class would be very difficult to mimic with computer generated pictures. This is why the approach presented in this paper should be of value for various other pathosystems. The drawback of the method is that the standard used is actually based upon the agreement of specialists, and is not really a true measurement of the disease severity. This is why it is important to develop the standard images through the use of a panel of experts with as much experience as possible to ensure the proposed standards are representative.

Acknowledgments

The authors thank D Le Floch and R Gosse for their technical assistance; the eight experts from INRA, CETIOM, and GEVES for having willingly agreed to rate hundreds of pictures of cankered cross-sections; Jean-Philippe Maigniel (GEVES) and the twenty assessor apprentices who kindly accepted the invitation to participate in the evaluation of Phomadidacte.

References

Aubertot, J.N., Schott, J.J., Penaud, A., Brun, H. & Doré, T. 2004: Methods for sampling and assessment in relation to the spatial pattern of phoma stem canker (*Leptosphaeria maculans*) in oilseed rape. – European Journal of Plant Pathology 110: 183-192.

Canteri, M.G. & Giglioti, E.A. 1998: Combro: software for selection and training people to estimate severity of sugarcane rust and borer-rot complex. – Summa Phytopathologica 24: 190-192.

Cooke, B.M. 1998: Disease assessment and yield loss. – In: Jones, D.G. (ed.). The Epidemiology of Plant Disease. Kluwer Academic Publishers, Dordrecht (The Netherlands): 42-72.

Doré, T., Sebillotte, M. & Meynard, J.M. 1997: A diagnostic method for assessing regional variations in crop yield. – Agricultural Systems 54: 169-188.

Gilligan, C.A. 1980: Size and shape of sampling units for estimating incidence of stem canker on oil-seed rape stubble in field plots after swathing. – Journal of Agricultural Science 94: 493-496.

Nutter, F.W., Gleason, M.L., Jenco, J.H. & Christians, N.C. 1993: Assessing the accuracy, intra-rater repeatability, and inter-rater reliability of disease assessment systems. – Phytopathology 83: 806-812.

Nutter, F.W. & Schultz, P. 1995: Improving the accuracy and precision of disease assessment: selection of methods and use of computer-aided training programs. – Canadian Journal of Plant Pathology 17: 174-184

O'Brien, R.D. & van Bruggen, A.H.C. 1992: Accuracy, precision, and correlation to yield loss of disease severity scales for corky root of lettuce. – Phytopathology 82: 91-96.

Pierre, J.G. & Regnault, Y. 1982: Contribution à la mise au point d'une méthode de plein champ destinée à mesurer la sensibilité des variétés de colza au phoma. – Informations Techniques du CETIOM 81(IV): 3-18.

Rempel, C.B. & Hall, R. 1996: Comparison of disease measures for assessing resistance in canola (*Brassica napus*) to blackleg (*Leptosphaeria maculans*). – Canadian Journal of Botany 74: 1930-1936.

Rimmer, S.R. & van den Berg, C.G.J. 1992: Resistance of oilseed brassica ssp. to blackleg caused by *Leptosphaeria maculans*. – Canadian Journal of Plant Pathology 14: 56-66.

SAS Institute. 1989: SAS/STAT User's Guide. Version 6. – SAS Institute, Cary, NC.

Tomerlin, J.R. & Howell, T.A. 1988: DISTRAIN: a computer program for training people to estimate disease severity on cereal leaves. – Plant Disease 72: 455-459.

van den Berg, C.G.J., Rimmer, S.R. & Parks, P. 1993: Comparison of scales for rating severity of blackleg in canola. – Canadian Journal of Plant Pathology 15: 49-53.

West, J.S., Kharbanda, P.D., Barbetti, M.J. & Fitt, B.D.L. 2001: Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. – Plant Pathology 50: 10-27.

Molecular detection of *L. maculans* and *L. biglobosa* spores from Burkard tapes

Anna Stachowiak, Witold Irzykowski and Małgorzata Jędryczka
Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland

Abstract: Oilseed rape in Poland is exposed to two fungal pathogens, *Leptosphaeria maculans* and *L. biglobosa*, which cause stem canker of cruciferes and are responsible for considerable yield losses. Infected stems that stay unploughed, remain on the soil surface and are the source of pseudothecia-fruiting bodies of the perfect stage that contain ascospores. Released ascospores are the main source of infection for young plants of winter oilseed rape in the following season. Information about timing of an ascospore release is a basis of decision support systems used to control the disease.

One of the standard tools used to detect timings of first and mass ascospore release, is a seven day volumetric spore trap by Burkard Manufacturing Ltd. (Rickmansworth, UK). Analysis of presence and concentration of airborne fungal spores may be performed either by means of conventional microscopy methods or PCR-based molecular techniques. In contrast to time consuming microscopy, PCR assays are faster and more accurate. This experiment was designed to optimise methods used for molecular detection of *L. maculans* and *L. biglobosa* spores from Burkard tapes. We have performed multiplex PCR using previously described primers, LmacA, LmacB and LmacRev as well as our own primers, LmF, LmR, LbF and LbR. Both Lmac, Lm and Lb primers were species specific. The primers worked very well in the presence of one of the two species. However, it was not possible to detect any spore DNA in case of imbalanced ratio between *L. maculans* and *L. biglobosa*. Freezing of a spore tape, which is done to store tapes for a long time, has increased the resolution of the method by 10 times. For *L. maculans*, the sensitivity of the method was ca. 8 spores on a frozen tape or fresh pycnidiospore suspension or 100 pycnidiospores, when the fresh tape was used for processing. The resolution for *L. biglobosa* was lower: 100 pycnidiospores from fresh and frozen tape. The detection of 4 and 10 pycnidiospores of *L. biglobosa* has been achieved, but the method was not reproducible.

Distribution and change in *L. maculans / L. biglobosa* populations in Poland (2000-2004)

Małgorzata Jędryczka and Elżbieta Lewartowska
Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland

Abstract: For four seasons (from 2000/01 to 2003/2004) we have collected fragments of oilseed rape plants with symptoms of infection with *Phoma lingam*. In 2000/01 samples were collected from 157 fields, in 2001/02 from 222 fields, 2002/2003 from 46 fields and in 2003/04 from 158 fields of winter oilseed rape. Sampling was done in most of the main areas of intensive cultivation of oilseed rape, including Lower Silesia, Opole region, West Pomerania and Pomerania with Zulawy (delta of Vistula river), Kujavia and Great Poland. Less intensive sampling was done in Lublin region, Varmia and Mazuria, Upper Silesia and the Lodz region located in central Poland. Sampling was done three times a year: in the autumn at rosette stage (symptoms on leaves), in the spring after start of vegetation extension (symptoms on leaves and stems) and before harvest (symptoms on stems). Pathogens were isolated after surface sterilisation with ethanol and calcium hypochlorite. In total, isolations resulted in 2841 fungal strains with 962, 256, 666 and 957 isolates for respective subsequent growing seasons. Isolates were characterised with colony morphology and pigment production on Czapek-Dox or Fries liquid media and identified as *Leptosphaeria maculans* and *L. biglobosa*. In general, half of the isolates belonged to one species and the other half to the other one. However, there were great differences between isolates from different seasons and years. The majority of isolates obtained in the autumn belonged to *L. maculans* with the average of 71.9% and a variation from 67.3 % in autumn 2003 to 8.2.6 % in autumn 2001. Spring samplings resulted in comparable numbers of isolates from either of the two species, with 55.7 % isolates of *L. maculans*. However, isolations from stems before harvest resulted in more *L. biglobosa* than *L. maculans* in a proportion reversed to this obtained in the autumn. Before harvest it was only 21.4 % of *L. maculans*, with variation from 16.9 % in summer 2001 to 25.9 % in summer 2003. Results obtained in this experiment support the hypothesis of possible reduction of *L. maculans* infections due to slow growth of the fungus in leaf tissues and petioles combined with leaf shearing due to frost damage. It is noteworthy, that in contrast to previous reports, *L. maculans* was detected in all regions of intensive cultivation of oilseed rape, including the east of Poland.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 259-264

Patterns of *Leptosphaeria maculans*/ *L. biglobosa* ascospore release in the season 2004/2005 in Poland

Joanna Kaczmarek[1], Agnieszka Maczynska[2], Idalia Kasprzyk[3], Andrzej Lewandowski[4] and Malgorzata Jedryczka[1]

[1] *Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland;* [2] *Institute of Plant Protection, Branch Sosnicowice, Gliwicka 29, 44-153 Sosnicowice, Poland* [3] *University of Rzeszow, Department of Environmental Biology, Cegielniana 12, 35-959 Rzeszow, Poland;* [4]*Research Centre for Cultivar Testing, 63-022 Slupia Wielka, Poland*

Abstract: Ascospores of *Leptosphaeria maculans* (Desm.) Ces. et de Not. and *L. biglobosa* Nov. are the main cause of stem canker of crucifers, one of the most damaging diseases of oilseed rape in Poland and worldwide. In the season 2004/2005 monitoring of airborne ascospores occurrence of these fungi was done in six regions of Poland (West Pomerania, Pomerania, Great Poland, Lower Silesia, Upper Silesia, Carpathian Foothills) within the framework of the System for Forecasting Disease Epidemics (SPEC). Spores were detected using 7-day volumetric spore traps or volumetric pollen and particle sampler surrounded by a circle of oilseed rape debris infected with *Leptosphaeria* spp. In West Pomerania, Pomerania and Lower Silesia monitoring was done from 1 September to 30 November 2004 and from 21 March to 7 June 2005. In Great Poland and Upper Silesia spore trapping has continued since 1 September 2004. In Carpathian Foothills, monitoring began in the spring 2005 and has continued to the present time. In autumn 2004, the difference between the date of the first spore detection in various regions was 12 days. Spores were first observed in Lower Silesia on 11 September 2004 and the latest date of detection was in Great Poland on 23 September 2004. The lowest numbers of spores were detected in Upper Silesia and the highest numbers (up to 320 spores/m^3 of air) in Lower Silesia. Lower Silesia had the longest duration of ascospore release (73 days from the detection of first spores in September to the detection of last spores in November 2004, including 54 days with spore release). In winter 2004/2005, no spores were captured either in Poznan (Great Poland) or in Sosnicowice (Upper Silesia). In spring 2005 ascospores were trapped at very low quantities only in Rarwino (West Pomerania), Tarnow (Lower Silesia) and Rzeszow (Carpathian Foothills).

Key words: Ascospore concentration, epidemic, *Leptosphaeria maculans*, *L. biglobosa*, spore trap, monitoring, stem canker

Introduction

The ascomycete fungi *Leptosphaeria maculans* (Desm.) Ces. et de Not. and *L. biglobosa* Nov. (Shoemaker & Brun, 2001) cause stem canker of crucifers, one of the most important diseases of oilseed rape in Poland and worldwide. Stem canker may result in plants necrosis and large yield losses (Zhou *et al*., 2000) especially if plants are infected by *Leptosphaeria maculans*, the more aggressive and damaging of the two species (Johnson & Lewis, 1994). In Poland, both *Leptosphaeria* species are present and the proportion of each depends on season, field location, history of cultivars grown, general and local weather and numerous other factors (Jedryczka & Lewartowska, 2006).

The main source of infection is ascospores produced in pseudothecia developing on infected stubble from a previous cropping season (Petrie, 1995). Intensity of pseudothecia

maturation and spore release depends on weather conditions to great extend. Pseudothecial development is abundant at temperatures from 5°C to 20°C (Toscano-Underwood *et al.*, 2003), and ascospores are released at the same range of temperatures (Huang *et al*., 2005). Ascospore release is also influenced by wetness. Small concentrations of ascospores in the air can be observed after dews (Huang *et al*., 2005), but ascospore showers are triggered by rainfall (Salam *et al*., 2003; Huang *et al*., 2005). Conducive conditions in summer and autumn lead to development of severe stem cankers epidemics (McGee, 1977; Hall, 1992).

Recently in Poland, the System for Forecasting Disease Epidemics (SPEC) has been developed to improve stem canker management (Jedryczka *et al.*, 2004). The aim of the system is to optimize timing of fungicide treatment, based on monitoring of pseudothecia maturation and ascospores presence in the air in different regions of Poland. SPEC aims to determine the periods when application of fungicides would be most effective, based on onset of pseudothecia maturation and release of ascospore showers (Jedryczka *et al.,* 2006).

The main aim of this experiment was to detect the first and subsequent major release of ascospores in five different regions covering the west and south of Poland, where oilseed rape is one of the most important crops.

Material and methods

Location of spore samplers

In season 2004/2005 monitoring of ascospore release was done using six spore traps located in six regions of intensive oilseed rape cropping in Poland. The regions were determined based on climatic zones of Poland (Wiszniewski & Chelchowski, 1987). The location of spore traps was as follows (Figure 1):

1. Lower Silesia - Experiment Station for Variety Testing in Tarnow;
2. Upper Silesia – Institute of Plant Protection, Branch Sosnicowice;
3. Great Poland – Institute of Plant Genetics in Poznan;
4. Pomerania - Experiment Station for Variety Testing in Radostowo;
5. West Pomerania – Experiment Station for Variety Testing in Rarwino;
6. Carpathian Foothills – University of Rzeszow, Krasne.

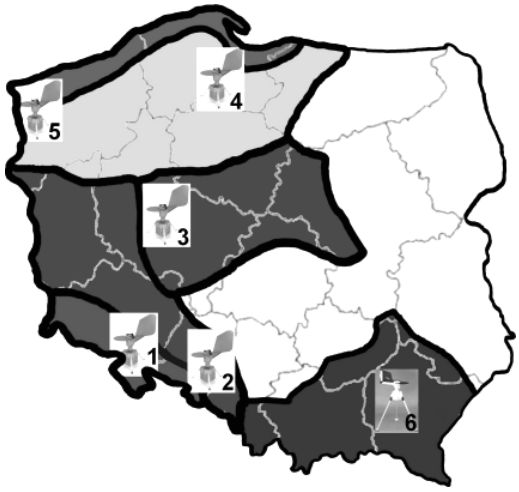


Figure 1. Climatic regions and location of spore traps used for monitoring spores concentration of Leptosphaeria spp. in Poland during the season 2004/2005; 1 - Lower Silesia (Tarnow), 2 - Upper Silesia (Sosnicowice), 3 - Great Poland (Poznan), 4 – Pomerania (Radostowo), 5 - West Pomerania (Rarwino), 6 – Carpathian Foothills (Krasne)

Sampling method

Investigation of *Leptosphaeria maculans* and *L. biglobosa* ascospore release was done using volumetric spore tarps. Monitoring of airborne ascospores presence was performed using either a seven-day volumetric trap manufactured by Burkard Manufacturing Ltd., UK (Regions 1-5) or a volumetric pollen and particle sampler produced by Lanzoni S.r.l., Italy (Region 6). Each spore trap was surrounded by infected oilseed rape stubble which had been collected at the end of the previous season and kept outdoors.

The mechanism of sampling of both Burkard and Lanzoni trap is the same. The samplers actively suck in a known volume of air. Spores, pollens and other airborne particles are impacted on a tape covered with an adhesive substance that covers a drum rotating at a constant speed of 2 mm per hour. After one week, tapes are removed and cut into seven equal pieces that represent 24 hours of sampling. A separate microscope slide is prepared for each piece of tape, the number of spores was counted and the concentration of spores per $1m^3$ of air per day was calculated.

Sampling periods

In West Pomerania, Pomerania and Lower Silesia monitoring was done from 1 September 2004 to 30 November 2004 and from 21 March 2005 to 7 June 2005. In Great Poland and Upper Silesia spore trapping has continued since 1 September 2004. In Carpathian Foothills, monitoring began on 20 April 2005 and has continued to the present time.

Results and discussion

In autumn 2004, ascospores were observed in all five regions but variation was observed both for periods of spore release, ascospore concentration and time of detection of peak spore number (Figure 2).

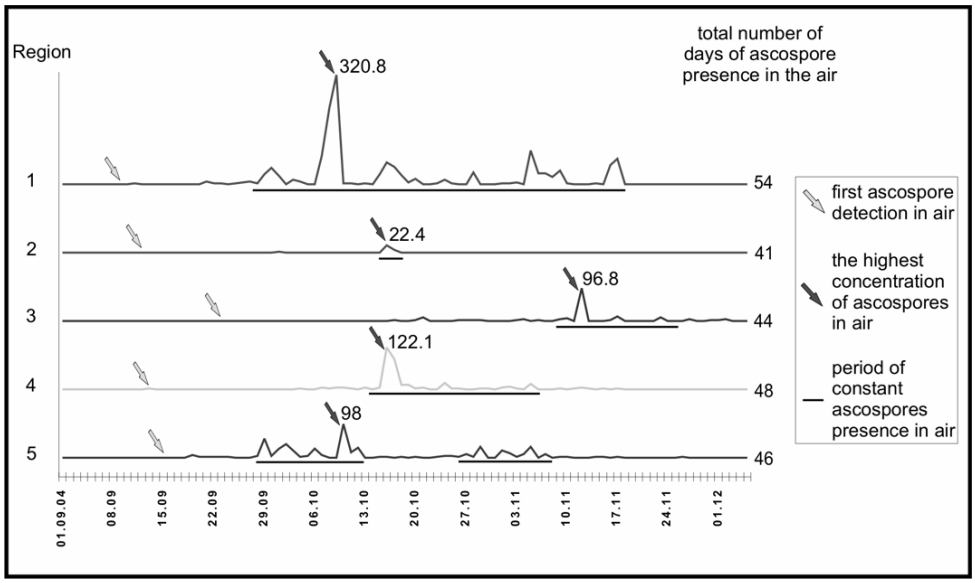


Figure 2. Concentrations and periods of airborne *Leptosphaeria maculans* and *L. biglobosa* ascospore presence in five locations of Poland in autumn 2004; 1 – Tarnow, 2 – Sosnicowice, 3 – Poznan, 4 – Radostowo, 5 – Rarwino

Time of first ascospore detection was similar in all regions studied where the difference ranged over 12 days. Earliest detection was in Lower Silesia (11 September) and the latest was in Great Poland (23 September). Ascospores of *Leptosphaeria maculans* and *L. biglobosa* were released at the highest intensity and for the longest period in Tarnow (Lower Silesia). The concentration of spores in the air in this region reached 320 spores/m^3/day and the number of days with a constant presence of spores was 54 (Figure 2). Ascospores were released at a high intensity in West Pomerania and Pomerania (north Poland), where concentration reached 96 spores/m^3/day and 122 spores/m^3/day, respectively. In these regions spores were observed for a similar period of time, 46 and 48 days respectively. In Great Poland, ascospores were observed for a similar period of time (46 days), but intensity of spore release was lower (4.2 spores/m^3/day on average). However, the number of spores peaked at 97 spores/m^3/day for a short period. In Upper Silesia, a low intensity of spore release was observed with the highest number of ascospores in air being 22/m^3/day and the average concentration for all 41 days when spores were observed was 1.1/m^3/day (Figure 2).

In spring 2005, ascospores of *Leptosphaeria maculans* and *L. biglobosa* were present in air samples of four of the six regions studied and only sporadically (Figure 3). Ascospores were detected at the highest number and for the longest period in the tectonic foreland, the areas of Lower Silesia and the Carpathian Foothills. Nevertheless, the number of spores did not exceed 3/m^3/day of air samples. In other regions, there were no airborne ascospores observed (Pomerania, Upper Silesia) or spore concentration was very low at a rate of 0.2/m^3/day (West Pomerania, Great Poland) (Figure 3).

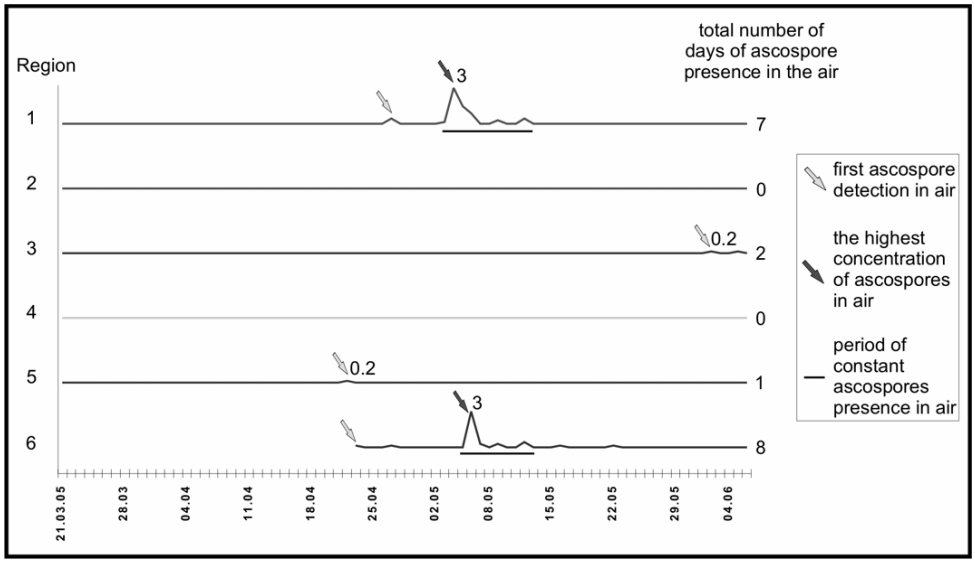


Figure 3. Concentrations and periods of airborne *Leptosphaeria maculans* and *L. biglobosa* ascospore presence in six locations of Poland in spring 2005; 1 – Tarnow, 2 – Sosnicowice, 3 – Poznan, 4 – Radostowo, 5 – Rarwino, 6 – Krasne

The results are in accordance with biology of *Leptosphaeria maculans* and *L.biglobosa* and with climatic zones of Poland. In autumn 2004, the highest concentrations of airborne ascospores were observed in Lower Silesia, Pomerania and West Pomerania. In July and August 2004 these three regions had the highest amount and frequency of precipitation in comparison to Great Poland and Upper Silesia. In the experiment located in Pomerania, the

total amount of rainfall in summer months exceeded 205 mm, and in Lower Silesia and West Pomerania rainfall was about 170 mm (Table 1). In the other two locations, the level of rainfall was as much as 50 mm less. A very dry period in the last 10 days of July and the first 10 days of August with nearly no rain (less than 5 mm rainfall in the period of 21 days) might result in a very late ascospore release in Poznan (Great Poland). Such dry conditions may have led to the complete drying of oilseed rape debris which resulted in the death of fungal mycelium and fruiting bodies and subsequently delayed the process of fungus maturation.

Table 1. Temperature and rainfall in places of spore trapping in Poland

Temperature (°C)																				
location	July				August				September				October				November			
	I	II	III	$\overline{X}$	I	II	III	$\overline{X}$	I	II	III	$\overline{X}$	I	II	III	$\overline{X}$	I	II	III	$\overline{X}$
1	16.4	17.7	18.4	17.5	18.9	20.5	16.6	18.7	14.6	14.6	11.6	13.6	11.8	6.00	12.2	10.0	7.30	3.20	1.90	4.10
2	17.8	18.4	19.3	18.5	19.4	20.3	17.0	18.9	14.6	13.9	10.5	13.0	-	6.7	12.4	9.6	7.3	-	1.3	4.3
3	17.0	17.6	19.1	17.9	21.6	21.1	16.4	19.7	14.7	14.5	11.6	13.6	12.1	7.1	11.2	10.1	7.0	3.7	2.1	4.3
4	15.6	15.6	17.4	16.2	19.8	18.9	16.4	18.4	14.8	14.3	11.6	13.5	11.3	6.5	9.7	9.2	5.8	3.4	0.9	3.4
5	15.5	16.3	17.5	16.4	20.3	19.4	15.8	18.5	13.7	13.9	11.9	13.2	11.0	7.2	10.4	9.5	6.6	3.7	2.4	4.2
Rainfall (mm)																				
location	July				August				September				October				November			
	I	II	III	∑	I	II	III	∑	I	II	III	∑	I	II	III	∑	I	II	III	∑
1	55.5	11.3	54.5	121.3	16.4	23.4	10.8	50.6	0.0	7.4	12.2	19.6	13.2	25.9	1.3	40.4	20.8	29.2	6.0	56.0
2	8.1	13.5	26.6	48.2	23.0	35.6	13.0	71.6	0.0	7.7	19.9	27.6	-	21.7	11.4	33.1	7.8	-	18.3	26.1
3	28.1	24.3	1.0	53.4	3.8	35.8	29.2	68.8	0.0	6.2	25.3	31.5	11.8	22.1	19.0	52.9	6.7	25.4	21.0	53.1
4	44.7	17.3	47.1	109.1	11.9	58.6	26.2	96.7	3.6	5.2	20.7	29.5	14.2	30.7	25.0	69.9	2.1	6.5	23.1	31.7
5	45.7	35.6	11.0	92.3	5.5	42.7	29.1	77.3	0.6	4.5	30.6	35.7	5.8	26.0	12.3	44.1	17.8	32.7	16.2	66.7

Explanations:
1 – Tarnow, 2 – Sosnicowice, 3 – Poznan, 4 – Radostowo, 5 – Rarwino
I, II, III – respective 10 days of particular months

Summer temperatures in experiment places located in Lower Silesia, Pomerania and West Pomerania were lower on average by 1-2 ^{o}C in July and 0.5-1 ^{o}C in August (Table 1). This means that a cumulative number of degrees differed on average by as much as 70 degrees. Cooler days and more precipitation surely had a strong effect on better survival, viability and a speed of maturation of the fungus on plant debris in the field after harvest in these three regions. In spring 2005 ascospores were released only sporadically and at very low concentrations. The reason for this was a long period of low temperatures and persistent snow cover during winter 2004/2005 in Poland.

Acknowledgements

We would like to acknowledge DuPont Poland for funding the project. We are very grateful to Prof. Edward Gacek, Director of COBORU, for permission to do spore sampling at experimental stations of the Research Station for Cultivar Testing (SDOO, COBORU). Special thanks for Anna Araszkiewicz from SDOO Rarwino, Teresa Sliwa from SDOO Radostowo, Albin Wojcicki from SDOO Tarnow for technical assistance with spore traps.

References

Hall, R. 1992: Epidemiology of blackleg of oilseed rape. – Canadian Journal of Plant Pathology 14: 46-55.

Huang, Y.J., Fitt, B.D.L., Jedryczka, M., Dakowska, S., West, J.S., Gladders, P., Steed, J.M. & Li, Z.Q. 2005: Patterns of ascospore release in relation to phoma stem canker epidemiology in England (*Leptosphaeria maculans*) and Poland (*Leptosphaeria biglobosa*). – European Journal of Plant Pathology 111: 263-277.

Jedryczka, M. & Lewartowska, E. 2006: Distribution and change in *L. maculans /L. biglobosa* populations in Poland (2000-2004). – IOBC/wprs Bulletin 29 (7): 257.

Jedryczka, M., Matysiak, R. & Graham, K. 2004: LeptoNet and SPEC - new projects supporting the control of stem canker of oilseed rape in Poland. – IOBC/wprs Bulletin 27 (10): 125-130.

Johnson, R.D. & Lewis, B.G. 1994: Variation in host range, systemic infection and epidemiology of *Leptosphaeria maculans*. – Plant Pathology 43: 269-277.

Kaczmarek, J., Jedryczka, M. & Czernichowski, J. 2005: Development of a decision support system for control of stem canker of oilseed rape in Poland. – Integrated Control in Oilseed Crops. Biannual Meeting, 11-12 Oct. 2005, Poznan, Poland. Abstract book: 35.

McGee, D.C. 1977: Blackleg (*Leptosphaeria maculans* (Desm.) Ces. et de Not.) of rapeseed in Victoria: Sources of infection and relationships between inoculum, environmental factors and disease severity. – Australian Journal of Agricultural Research 28: 53-62.

Petrie, G.A. 1995: Patterns of ascospore discharge by *Leptosphaeria maculans* (blackleg) from 9- to 13-month-old naturally infected rapeseed/canola stubble from 1977 to 1993 in Saskatchewan. – Canadian Plant Disease Survey 75: 35-43.

Salam, M.U., Khangura, R.K., Diggle, A.J., & Barbetti, M.J. 2003: Blackleg Sporacle: A model for predicting onset of pseudothecia maturity and seasonal ascospore showers in relation to blackleg of canola. – Phytopathology 93: 1073-1081.

Shoemaker, R.A. & Brun, H. 2001: The teleomorph of the weakly aggressive segregate of *Leptosphaeria maculans*. – Canadian Journal of Botany 79: 412-419.

Toscano-Underwood, C., Huang, Y.J., Fitt, B.D.L. & Hall, A.M. 2003: Effects of temperature on maturation of pseudothecia of *Leptosphaeria maculans* and *L. biglobosa* on oilseed rape stem debris. – Plant Pathology 52: 726-36.

Wiszniewski, W. & Chelchowski, W. 1987: Regiony klimatyczne. – In: Atlas hydrologiczny Polski. Wydawnictwa Geologiczne, Warszawa.

Zhou, Y., Fitt, B.D.L., Welham, S.J., Evans, N. & Gladders P. 2000: Effect of stem canker (*Leptosphaeria maculans*) and leaf spot (*Pyrenopeziza brassicae*) on yield of winter oilseed rape (*Brassica napus*) in southern England. – Plant Pathology 49: 487-497.

Polymorphisms within *Leptosphaeria maculans* and *Leptosphaeria biglobosa* relvealed with rep-PCR fingerprints

Witold Irzykowski and Małgorzata Jędryczka
Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland

Abstract: The method of rep-PCR was originally designed for species or strain differentiation of prokaryotes, but later it was successfully used to generate DNA fingerprings for numerous fungi, including '*Leptosphaeria maculans* species complex'. The method allows amplification of sequences between randomly dispersed repetitive sequences in a genome and it proved its usefulness in discriminating different components of the species complex and in evaluating a level of polymorphism within members of this complex. In a current study, 200 isolates, including 111 isolates of *L. biglobosa* and 89 isolates of *L. maculans*, were studied using primers derived from the 'repetitive extragenic palindromic' (REP) sequence, the 'enterobacterial repetitive intergenic consensus' (ERIC) sequence and the conserved repeated DNA element 'BOX'. In *L. biglobosa*, two polymorphic bands were found for REP and three polymorphic bands were found for both ERIC and BOX. Surprisingly, more variation was found for *L. maculans*, with five polymorphic bands for REP, twenty nine for ERIC and three for BOX. This polymorphism was not connected with any known character of two species in study. Isolates differing from by a substitution in ITS1-5.8S-ITS2 region did not form any specific group with a unique rep-PCR fingerprint. Different fingerprints were not specific to geographical location.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 267-276

Development of a decision support system for control of stem canker of oilseed rape in Poland

Malgorzata Jędryczka [1], Joanna Kaczmarek [1] and Jacek Czernichowski [2]
[1] Institute of Plant Genetics, Polish Academy of Sciences, 60-479 Poznan, Poland [2] DuPont Poland, 01-797 Warszawa, Poland

Abstract: System for Forecasting Disease Epidemics (SPEC; System Prognozowania Epidemii Chorób) is a decision support system for improved control of stem canker of oilseed rape in Poland. The system is a joint initiative between the Institute of Plant Genetics PAS and DuPont Poland. Activities of the system are supported by several research organisations, such as the Institute of Plant Protection, Central Cultivar Testing Station, University of Rzeszow, Agricultural University of Poznan, Institute of Soil Science and Plant Cultivation and two commercial enterprices: Arenda in Charbielin and Agro-Fundusz Mazury in Drogosze.

Stem canker of crucifers is a serious disease of oilseed rape in Poland and worldwide. The disease is caused by the ascomycete fungi *Leptosphaeria maculans* and *Leptosphaeria biglobosa*. SPEC aims to optimise fungicide sprays against stem canker. To achieve this, the system uses a series of seven day volumetric spore traps (Burkard Manufacturing, UK and Lanzoni, Italy) to monitor the concentration of fungal ascospores in the air. The system has been operating since 1 September 2004 with five spore traps operating during the 2004/05 season and ten traps in the 2005/06 season. The traps are located across the main oilseed rape growing areas of Poland, namely Lower Silesia, West Pomerania, Pomerania and west Varmia, Great Poland, Upper Silesia, the north part of the Opole region, the south part of the Opole region, the Carpathian Foothills, Mazuria with east Varmia and the area encompassing the Mazovia Plain and Lublin region. Data on ascospore release are published on the websites of the SPEC system (www.spec.edu.pl) and DuPont Poland (www.dupont.pl). The results are updated weekly from September to November and from March to May. Information is also distributed free of charge *via* e-mail, SMS text messages or fax to registered users.

Since the beginning of the 2005/2006 season, a network of sampling points to examine maturity of fungal fruiting bodies on infected straw has been established. Monitoring of pseudothecia maturity is performed in 44 locations and covers all provinces of Poland.

To investigate the efficiency of fungicide treatments an experiment has been done with the timing of application being based on spore sampling results. In parallel, monitoring is accompanied by two field experiments located in regions with different weather conditions located *ca*. 330 kilometres apart.

The target end users of SPEC are oilseed rape farmers, breeders, extension services and the commercial companies who distribute agrochemicals.

Key words: Oilseed rape, stem canker of crucifers, forecasting disease epidemics, ascospore release, spore samplers.

Introduction

In Poland, oilseed rape is cultivated annually on *ca*. 500 thousand hectares and it is forecasted that soon the area under this crop will greatly expand due to biofuel production (Rosiak, 2005). Due to adverse weather conditions – harsh winters in east of the country, and other circumstances such as distance from the nearest oil pressing plant, the crop is unevenly distributed across the country. At present it is mainly grown in the west of the country, including the regions of Pomerania, West Pomerania, Great Poland and Gorzow Region,

Lower Silesia and Opole Region and some central and north-east parts of the country, including Kujavia, Varmia and Mazuria regions and the production of oilseed rape in these regions continues to increase. However, demand for seed has encouraged expansion of the crop to occupy new regions of Poland, including other central and eastern regions of the country with Upper Silesia, the Carpathian Foothills, the Mazovia Plain and Lublin regions as the leading ones.

High intensity of oilseed rape production, large fields, more often use of rapeseed in crop rotation and milder winters make the crop more prone to fungal pathogens. The two main fungal diseases of oilseed rape in Poland are stem canker of crucifers caused by '*Leptosphaeria maculans* species complex' and sclerotinia stem rot caused by *Sclerotinia sclerotiorum*. The former is observed every year and over the whole of Poland (Jedryczka & Lewartowska 2006) whereas the latter is observed mainly in western regions of the country in years with a wet spring. In Poland, stem canker of crucifers and stem lesions are observed primarily on winter oilseed rape, whereas sclerotinia stem rot is observed on both winter and spring crops (Jedryczka *et al.* 1999). Currently *L. maculans* does not cause economical problems on spring oilseed rape (Sadowski *et al*., 2002). It is noteworthy that the population of the fungus in Poland encompasses two fungal species: *Leptosphaeria maculans* (Desm.) Ces. et de Not. and *L. biglobosa* identified by Shoemaker & Brun (2001) (Jedryczka & Lewartowska, 2006).

Stem canker is responsible for considerable yield losses. Therefore an early detection of plant infection and the identification of causal agents are important components of protection against the disease (West *et al*. 2001). The main source of plant infection are ascospores that are produced in pseudothecia - fruiting bodies of the generative stage (*Leptosphaeria* sp.). The use of spore traps to monitor ascospore presence and concentration in air samples makes it possible to pinpoint periods of early plant infections. It has been suggested that early plant infection causes greater yield loss (Zhou *et al.,* 1999, Sun *et al*., 2000). Hence, the detection of early infections and detection of low levels of infections are both important elements to combat the disease. At present, systems of monitoring ascospore concentration of *L. maculans* operate in the UK (SPAWS [http://www.syngenta-crop.co.uk/spaws/] a component of the PASSWORD project: http://phoma.csl.gov.uk) (Sutherland *et al.* 2002, Gladders *et al.* 2004 and 2006), Australia (BLACKLEG SPORACLE: http://www.agric.wa.gov.au) (Salam *et al*. 2003) and Poland (SPEC: http://www.spec.edu.pl). The Polish system, called the System for Forecasting Disease Epidemics (*pol.* System Prognozowania Epidemii Chorob, acronym: SPEC, translated in English as EXPERT) began in autumn 2004 (Jedryczka *et al*. 2004). In the first year of activity, the System covered western regions of the country. Since autumn 2005 the System has greatly expanded and at present it covers the whole of Poland.

Materials and methods

General information

The System for Forecasting Disease Epidemics (SPEC) began operation on 1 September 2004. In the 2004/2005 season, SPEC encompassed six geographical locations in different climatic regions, primarily in west Poland. To begin with, SPEC covered five regions (Pomerania and West Pomerania, Great Poland, Lower Silesia and Upper Silesia) and another site, in the Carpathian Foothills (located in south-east Poland) was added in spring 2005. At each location we collected data on the maturation of fruiting bodies on infected stems and on the concentration of ascospores in air samples. The system expanded in the summer 2005 and at present it covers all climatic regions of Poland with 44 stubble sampling sites and 10 spore traps.

Assignation of climatic regions

At present SPEC covers all climatic regions of Poland that were described by Wiszniewski & Chelchowski (1987) who divided Poland into fourteen climatic regions. The location of spore traps throughout these regions is shown in Figure 1. SPEC regions no. 2, 6 and 10 are composed of two or three climatic regions assigned by Wiszniewski and Chelchowski (as explained in Table 1). In contrast, regions 4, 5, 7 and 8 represent the climatic division between the regions of Great Poland and Mazovia (into separate region of Great Poland and separate region of west Mazovia and Kujavia) and the Sudethian Foothills (into a separate region encompassing the southern part of Lower Silesia and south part of Opole). Location of two spore traps at different locations in these climatic regions was caused by high intensity of oilseed rape production in these zones.

Evaluation of pseudothecia maturation

All 10 spore traps were surrounded by comparable amounts of infected oilseed rape stubble collected the previous season in the climatic region of trap location. Moreover, there are 34 extra sampling sites of infected stubble that help to evaluate the rate of pseudothecial maturation process (Figure 2).

Stubble fragments are maintained under natural conditions outdoors so that fruiting bodies of the fungus that had previously infected the stubble are subjected to natural weather conditions such as rainfall and temperatures characteristic for that specific region and they therefore mature at a comparable speed to those on farmers' fields. The development of the pseudothecial maturation process is monitored at weekly intervals. Six to ten stem fragments with visible fruiting bodies were collected at each site and sent by priority mail to the Institute of Plant Genetics PAS and observed under a light microscope not later than two days after arrival of the sample. Fruiting bodies are divided into pycnidia (anamorph stage, producing pycnidiospores) and pseudothecia (teleomorph, producing ascospores). Pseudothecia are divided into five classes of maturation:

- class A – immature pseudothecia;
- class B – pseudothecia with forming asci;
- class C – ascospores present in asci, but immature (less than 6 cells) and/or less than 8 ascospores in one ascus;
- class D – 8 fully mature ascospores in each ascus;
- class E – pseudothecia mature, but empty after discharge of ascospores.

In total 60 fruiting bodies are observed every week at each sampling site. The names of all 44 sampling sites and their locations are presented in Table 1. Out of 44 locations, 10 of the pseudothecial maturation sampling sites correspond to 10 of the ascospore trap locations and the remaining pseudothecial maturation sampling sites are located in other parts of the climatic regions or in the borderline areas between these regions. By locating sampling sites at several places within each region, we are able to check the uniformity or differences in maturation rate within regions. In addition, sites in the border between adjacent regions allow us to monitor for differences in disease risk between regions and help to decide what type of information should be directed to farmers whose fields are located close to a particular edge of the region. Also, 10 sampling sites are located in Great Poland close to each other, to check the level of similarity (or dissimilarity) of information gathered at different sampling places located within the same climatic region.

Evaluation of ascospore concentration in air samples

Due to higher intensity of oilseed rape production in north, south-west and some central regions of the country, more spore traps are located in these regions. In total we operate ten spore traps including nine seven-day volumetric Burkard spore traps (Burkard Manufacturing

Ltd., UK) and one volumetric pollen and particle sampler produced by Lanzoni S.r.l. (Italy). Both traps, although produced by two different companies, use a very similar system of collecting air samples, so the results can be directly compared. Spore samplers contain a built-in vacuum pump, designed to sample airborne particles such as fungus spores and pollens. The air is actively sampled at a volume of 10 litres per minute. Particles are impacted on adhesive coated transparent plastic tape supported on a clockwork-driven drum. After each week tapes are cut longitudinally in two halves and transversely in seven equal parts representing seven days (24 hour periods) of each week. One half of the tape for each sampling date is stained and mounted as a permanent microscope slide and is then analysed under a light microscope. The second half of the tape for each sampling date is cut into smaller pieces and placed in a sterile Eppendorf tube for molecular analysis. The samples are collected from 1 September to 30 November and from 1 March (or later if snow cover persists) to 31 May, and sent to IPG for assessment at week intervals. All samples are sent to IPG immediately after preparation and then ascospores of *L. maculans* and *L. biglobosa* are observed and counted. The result is re-calculated for the number of ascospores in a cubic metre of the air. The results of the microscopic analysis are published on two websites: the commercial website of DuPont Poland (www.dupont.pl) and on an educational website of the project (www.spec.edu.pl) maintained by IPG. On the DuPont website, the results are shown in the form of a map showing the level of risk of plant infection, separately for 12 climatic zones shown in Figure 1 and also in a form of short communications. On the IPG-maintained website, we show the same map as published on the DuPont website and more detailed results in the form of graphs (a curve of ascospore concentration in air samples over time and percent of pseudothecia of each maturity class for all twelve regions). The level of risk is encoded using three colours: green – for no risk, yellow for increased risk and red for a high risk. The increased risk is denoted by the time when the first ascospores are detected in air samples and a high risk situation is announced *ca*. two weeks after the detection of the first ascospores in air samples or in the case of the detection of high levels of ascospores.

Collection of meteorological data

All sampling sites with spore samplers except these in Drogosze (Region 3) and Charbielin (Region 8) have a dedicated meteorological station which records daily rainfall and temperature data (mean, minimum and maximum). The data for regions 3 and 8 are collected from the local station of the Institute of Meteorology and Water Management (IMGW) located ca. 20-30 km from the spore trapping site. The oilseed rape stubble sampling sites also have automatic meteorological stations or in some cases, meteorological data are available from nearby companies or stations of the IMGW.

Information delivery to farmers/target users

Farmers, representatives of services for farmers, fungicide distributors, breeders of oilseed rape, researchers, students and other people interested in results of monitoring are informed using several different methods: 1/ internet – websites: www.dupont.pl ; www.spec.edu.pl ; 2/ SMS text messages; 3/ emails; 4/ letters/faxes; 5/ farmers' press; 6/ conferences; 7/ practical sessions.

All information is provided free of charge. SMS text messages, emails, letters and faxes are provided to all persons who have shown interest in the results of SPEC and have provided their address which are stored in a database.

Table 1. The location of spore traps in climatic regions of Poland

No of a climatic region	Name of a climatic region [1]	Spore sampler location [2]	No of stubble sampling sites	Location of stubble sampling sites
1	Along the Baltic sea (Nadmorski)	No 1 (B) Rarwino	2	Kodrab, Rarwino
2	Pomerania Lake District (Pojezierze Pomorskie)	No 2 (B) Radostowo	1	Radostowo
3	Pomerania and Varmia Region (Pomorsko-Warminski)		2	Chrzastowo, Dolaszewo
4	Mazuria and Bialystok Region (Mazursko-Bialostocki)	No 3 (B) Drogosze	5	Biala Piska, Drogosze, Lyski, Suwalki, Wrocikowo
5a	Great Poland and Mazovia Region (Wielkopolsko-Mazowiecki)	No 4 (B) Poznan (Great-Poland)	10	Baborowko, Cerekwica, Działyń, Jerka, Jeziory, Pawłowice, Poznan, Strzałkowo, Szczodrzykowo, Turew
5b		No 5 (B) Glebokie (west Mazovia and Kujavia)	4	Glebokie, Radzikow, Skierniewice, Walewice
6	Mazovia and Podlasie Region (Mazowiecko-Podlaski)	No 6 (B) Pulawy	1	Siedlce
7	Lodz and Wielun Region (Lodzko-Wielunski)		3	Koscierzyn, Maslowice, Radom
8	Lublin and Zamosc Region (Lubelsko-Zamojski)		3	Bezek, Lesniowice, Pulawy
9a	Sudethian Foothills (Sudecki)	No 7 (B) Tarnow (south part of Lower Silesia)	2	Tarnow, Zloty Potok
9b		No 8 (B) Charbielin (south part of Opole Region)	1	Charbielin
10	Gorzow and Lower Silesia Region (Lubusko-Dolnoslaski)	No 9 (B) Sosnicowice	5	Malyszyn, Namyslow, Sosnicowice, Szprotawa, Zory,
11	Krakow and Sandomierz Region (Krakowsko-Sandomierski)	No 10 (L) Krasne	1	Sledziejowice
12	Carpathian Foothills (Karpacki)		1	Krasne
13	Krakow and Czestochowa Upland (Wyżyna Krakowsko-Czestochowska	Lack of a spore sampler	2	Sosnowiec-Porabka, Zawada
14	Swietokrzyskie Mountains (Gory Swietokrzyskie)	Lack of a spore sampler	1	Modliszewice

[1] Name of the climatic region according to Wiszniewski and Chelchowski (1987)
[2] Number of spore sampler according to Figure 1

(B) – Burkard spore sampler
(L) - Lanzoni spore sampler

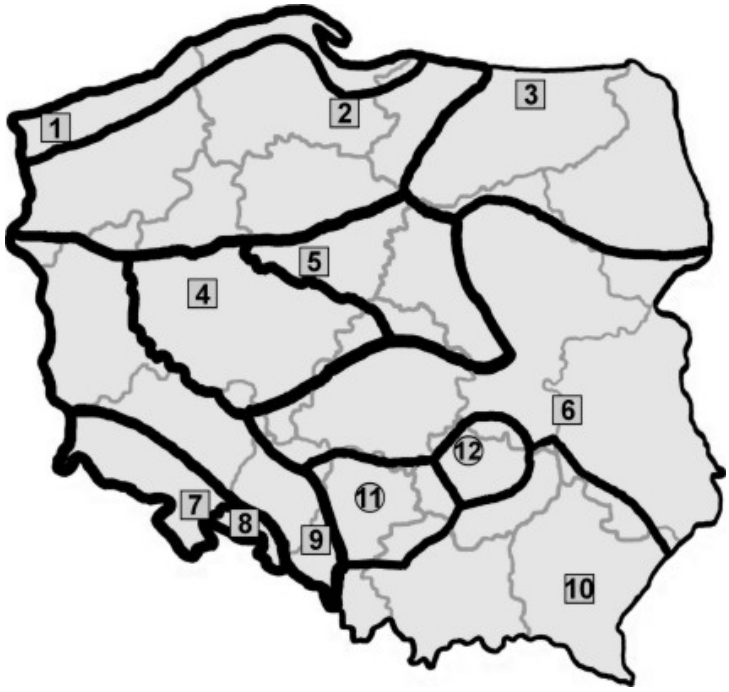


Figure 1. The climatic regions of the System for Forecasting Disease Epidemics (SPEC) in Poland. Numbers in squares indicate location of spore traps and they correspond to spore sampler location numbers in Table 1. Monitoring in regions 11 and 12 is based on evaluation of pseudothecial maturation on rapeseed stubble

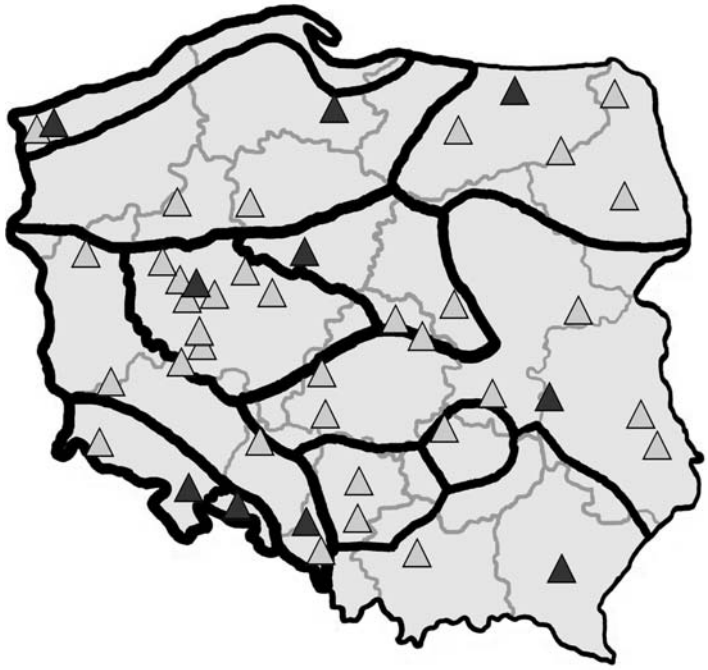


Figure 2. The location of stubble collection sites of the System for Forecasting Disease Epidemics (SPEC) in Poland. Black triangles correspond to sites with spore traps, grey figures correspond to sites with oilseed rape stubble only

Results and discussion

Since opening (April 2005), the SPEC website (www.spec.edu.pl) has been visited more than 4500 times. Visitors came from 12 of the 16 regions of Poland. There are *ca*. 15 visitors per day on average. However, if we take into account that the interest in results of the System is generally during the two month period from the middle of September to the middle November , this means that in real terms the site has 75 visitors per day. As the results of monitoring may also be of interest to farmers and researchers from neighbouring countries (we already have visitors from at least seven countries besides Poland), the website will be available in English from 2006 onwards.

The results obtained from SPEC was distributed by 1200 SMS text messages in 2004 and this number increased to 2500 messages during 2005. More than 1000 emails were sent in 2004, and in 2005 this number was doubled. At the begining of the project, producers of oilseed rape, services for farmers and distributors of fungicides were also informed about the results by post (*ca*. 3500 in 2004). In 2005 the number of letters sent decreased to 2000, as more end users registered for emails and SMS text communication. This trend indicates that more farmers purchased and/or learnt how to use computers and more of them began to use mobile phones. This tendency to use new technologies for communication is a bonus for the SPEC system as the most up-to-date information is able to be accessed by recipients earlier and this allows more time for decision making with respect to method/timing of crop protection. We have also published several research articles about SPEC; some of these contained general information on the organisation of the System and some have presented detailed results. Information from SPEC has also been distributed to numerous farmers *via* conferences organised by research institutes and also through field visits for farmers.

The questionnaires sent to farmers in autumn 2005 (after the first year of the project) revealed that as many as 96 % of farmers had knowledge of the existence of SPEC and the 41 % of these farmers took into consideration the results of SPEC when making crop protection decisions. However, it should be stressed that this feedback came from farmers on a database of DuPont and that these growers are therefore used to participating in conferences, seminars and practicals organised by the company. It is difficult to ascertain a realistic number for the total percentage of Polish farmers that grows oilseed rape and that are aware of SPEC. Most farmers (55 %) learned about SPEC from one of the farmers conferences organised by DuPont, or from DuPont representatives or the farmers' press (41 % each possibility). A popular way of technology transfer (23 % of farmers) was *via* "field days" organized as practical sessions in demonstration fields. A small percentage of farmers (2 %) became aware of SPEC through information from farm advisors. When asked how crop protection decisions were made indicated that they based decisions on observations of disease symptoms in the field (78 %), or on information from SPEC (39 %). Others made decisions following contact with representatives of a distribution service (26 %). About one fifth of farmers (22 %) applied chemical sprays on a calendar date each year. Both websites are of similar importance for internet users with 55 % of visitors visiting the DuPont website and 45 % having visited the educational website maintained by IPG. About one third of persons visited a website several times, one third a few times and the other third visited once. Most of the farmers who responded to the questionnaire grow rapeseed every year (96 %) for an average 18 years (ranging from 1 year minimum to 35 years maximum). The average field size was more than 100 hectares with a maximum of 820 hectares. Considering the very small average size of farms in Poland (8.4 ha) it can be concluded that rapeseed is mainly grown on medium to large farms.

To our knowledge, the SPEC system is currently the largest system of monitoring pseudothecia maturation and ascospore release in the world, followed by Blackleg Sporacle in Australia and SPAWS, a component of the PASSWORD project in the UK. SPEC gathers more than 4900 data, including ca. 4000 data on pseudothecia maturation and 900 data on ascospore release. This information is gathered two times per year, in autumn (September-November) and in spring (March-May). Moreover, as four of the ten spore traps operate all year round, additional information is available from four locations (central-west, central south, south-east and central-east Poland). Data on pseudothecia maturation, ascospore concentration in air samples and the meteorological data are collected and used for comparative studies of fungal development in different weather conditions, for epidemiological studies and modelling of the life cycle of the pathogens (West *et al*,. 2004, Huang *et al*., 2005, Salam *et al*., 2006, Aubertot *et al*., 2006, Kaczmarek *et al*., 2006). The results are compared with studies performed by other authors (Xu *et al*., 1987, Hershman & Perkins 1995, Thürwächter *et al*., 1999, Guo & Fernando 2005). Results of monitoring are also used in field experiments using fungicide treatments in arbitrarily chosen dates and periods pinpointed by the System. The two first years of activity of SPEC are very encouraging.

The fungal species *L. maculans* and *L. biglobosa* appear in different intensity and ratios (Jedryczka & Lewartowska, 2006). They are responsible for different severities of the disease (Williams & Fitt, 1999: West *et al*., 2004) and show different sensitivity to biologically active compounds (Eckert *et al.,* 2004), what causes difficulties in interpretation of some results. Therefore, current experimental work is directed at the detection of airborne inoculum using molecular methods which allow us to identify the composition of fungal inoculum in multiplex PCR reactions (Calderon *et al*., 2002). However, it was shown that the detection of fungal DNA of both species is not possible if the proportion between the two species differs more than 10 fold, especially if *L. maculans* dominates over *L. biglobosa* (Stachowiak *et al*., 2006). Using primers specific to both species it was demonstrated that the former species was easier to detect even if the ratio between the species was 10-fold in favour of *L. biglobosa*. With respect to understanding the disease processes affecting both species, there is a need to have working quantitative PCR system for analysing spore tapes.

SPEC is constantly being improved. At present, a new project has begun to investigate what recommendations should be made in a situation when an oilseed rape field located close to a particular trap (eg. *ca*. 100 km) is considered with respect to the results from a trap located much further away (eg. *ca.* 200-300 km) but located in the same climatic region. The results of monitoring are used to decide on current risk recommendations., However, the goal of the project is to create a mathematical model of pseudothecial maturation and ascospore release that would work in Polish weather conditions and that can be used without the support of microscopy or molecular experiments (Aubertot *et al*., 2006).

Acknowledgements

The authors thank to Andrzej Lewandowski who coordinates the activities of participants from the Research Centre for Cultivar Testing, Zbigniew Karolewski from the Agricultural University in Poznan, Idalia Kasprzyk from the University of Rzeszow and Anna Podlesna from the Institute of Soil Science and Plant Cultivaton in Pulawy for their help in the expansion of SPEC to eastern parts of Poland. Special thanks to Jaroslaw Grocholski from the Commercial and Service Company “Arenda” in Charbielin and Marek Kluczynski – the manager of “Agro Fundusz Mazury” for the expansion of the monitoring system to production fields of both companies in south-western (Opole Region) and north-eastern

(Mazuria) Poland. We are also very thankful to Neal Evans from Rothamsted Research for a thorough language correction of the manuscript.

References

Aubertot, J.N., Salam, M.U., Diggle A.J., Dakowska, S. & Jedryczka, M. 2006: SimMat, a new dynamic module of Blackleg Sporacle for the prediction of pseudothecial maturation of *L. maculans / L. biglobosa* species complex. Parameterisation and evaluation in Polish conditions. – IOBC/wprs Bulletin 29(7): 277-285.

Calderon C., Ward, E., Freeman, J., Foster, S.J. & McCartney, H.A. 2002: Detection of airborne inoculum of *Leptosphaeria maculans* and *Pyrenopeziza brassicae* in oilseed rape crops by polymerase chain reaction (PCR) assays. – Plant Pathology 51: 303-310.

Eckert, M.R., Fitt, B.D.L. & Selley, A. 2004: *Leptosphaeria maculans*, *L. biglobosa* and fungicides: preliminary results from *in vitro* and winter oilseed rape experiments. – IOBC/wprs Bulletin 27 (10): 157-164.

Gladders, P., Evans, N., Fitt, B.D.L., Walters, K., Turner, J., Northing, P., Sutherland, K., Campbell, S., Selley, S., Hall, B., Ellerton, B. & Naylor D. 2004: The PASSWORD project: a decision support system for managing pests and diseases of winter oilseed rape in the UK. – IOBC/wprs Bulletin 27 (10): 31-38.

Gladders, P., Evans, N, Marcroft, S. and Pinochet, X. 2006: Dissemination of information about management strategies and changes in farming practices for the exploitation of resistance to *Leptosphaeria maculans* (phoma stem canker) in oilseed rape cultivars. – European Journal of Plant Pathology 114: 117-126.

Guo, X.W. & Fernando W.G.D. 2005: Seasonal and diurnal patterns of spore dispersal by *Leptosphaeria maculans* from Canada stubble in relation to environmental conditions. – Plant Disease 89: 97-104.

Hershman, D.E. & Perkins D.M. 1995: Etiology of canola blackleg in Kentucky and seasonal discharge patterns of *Leptosphaeria maculans* ascospores from infested canola stubble. – Plant Disease 79: 1225-1229.

Huang, Y.J., Fitt, B.D.L., Jedryczka, M., West, J.S., Gladders, P., Steed, J.M. & Li, Z.Q. 2005: Patterns of ascospore release in relation to phoma stem canker epidemiology in England (*Leptosphaeria maculans*) and Poland (*Leptosphaeria biglobosa*). – European Journal of Plant Pathology 111: 253-277.

Jędryczka, M., Lewartowska, E. & Dakowska, S. 1999: Ocena podatnosci odmian rzepaku jarego na zgnilizne twardzikowa [Evaluation of susceptibility of spring oilseed rape cultivars to sclerotinia stem rot]. – Rośliny Oleiste [Oil Crops] 20 (2): 659-668.

Jedryczka, M. & Lewartowska, E. 2006: Distribution and change in *L. maculans / L. biglobosa* populations in Poland (2000-2004). – IOBC/wprs Bulletin 29 (7): 257.

Jedryczka, M., Matysiak, R. & Graham, K. 2004: LeptoNet and SPEC - new projects supporting the control of stem canker of oilseed rape in Poland. – IOBC/wprs Bulletin 27 (10): 125-130.

Kaczmarek, J., Maczynska, A., Kasprzyk, I., Lewandowski, A. & Jedryczka, M. 2006: Patterns of *Leptosphaeria maculans*/ *L. biglobosa* ascospore release in the season 2004/2005 in Poland. – IOBC/wprs Bulletin 29 (7): 59-64.

Rosiak, E. 2005: Produkcja rzepaku w Polsce i na swiecie [Production of oilseed rape in Poland and worldwide]. – In: Cz. Musnicki, I. Bartkowiak-Broda & M. Mrowczynski (eds.). Technologi produkcji rzepaku [Technology of oilseed rape production]. Wies Jutra, Warsaw.

Sadowski, C., Dakowska, S., Lukanowski, A. & Jedryczka, M. 2002: Occurrence of fungal diseases on spring rape in Poland. – IOBC/wprs Bulletin 25 (2): 1-12.

Salam, M.U., Fitt, B.D.L., Aubertot, J.N, Diggle, A.J., Huang Y.J., Barbetti, M.J., Gladders, P., Jedryczka, M., Khangura, R.K., Wratten, N., Fernando, W.G.D., Pinochet, X., Penaud, A. & Sivasithamparam K. 2006: Two weather-based empirical models for predicting onset of seasonal release of ascospores of *Leptosphaeria maculans* or *L.biglobosa,* causes of oilseed rape phoma stem canker in Australia, Canada, France, Poland or the UK. – Plant Pathology: (submitted).

Salam, M.U., Khangura, R.K., Diggle, A.J., & Barbetti, M.J. 2003: Blackleg Sporacle: A model for predicting onset of pseudothecia maturity and seasonal ascospore showers in relation to blackleg of canola. – Phytopathology 93: 1073-1081.

Shoemaker, R.A. & Brun, H. 2001: The teleomorph of the weakly aggressive segregate of *Leptosphaeria maculans*. – Canadian Journal of Botany 79: 412-419.

Stachowiak, A., Irzykowski, W. & Jedryczka, M. 2006: Molecular detection of *L. maculans* and *L. biglobosa* spores from Burkard tapes. –IOBC/wprs Bulletin 29 (7): 255.

Sun, P., Fitt, B.D.L., Gladders, P. & Welham, S.J. 2000: Relationships between phoma leaf spot and development of stem canker (*Leptosphaeria maculans*) on winter oilseed rape (*Brassica napus*) in southern England. – Annals of Applied Biology 137: 113-125.

Sutherland, K.G., Evans, N., Fitt, B.D.L., Gladders, P., Morgan, D., Northing, P., Turner, J. A., Walters, K.F.A. & Parker, C. 2002: Development of a DSS for diseases and pests in winter oilseed rape. – Proceedings Crop Protection in Northern Britain 2002: 163-168.

Thürwächter, F., Garbe, V. & Hoppe, H.H. 1999: Ascospore discharge, leaf infestation and variations in pathogenicity as criteria of predict impact of *Leptosphaeria maculans* on oilseed rape. – Journal of Phytopathology 147: 215-222.

West, J.S., Jedryczka, M., Penaud, A. & Fitt, B.D.L. 2004: Comparing fungal diseases on oilseed rape in England, France and Poland. – IOBC/wprs Bulletin 27 (10): 39-44.

West, J.S., Kharbanda, P.D., Barbetti, M.J. & Fitt, B.D.L. 2001: Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. – Plant Pathology 50: 10-27.

Wiszniewski, W. & Chelchowski, W. 1987: Regiony klimatyczne [Climatic regions]. – In: Atlas hydrologiczny Polski [Hydrological atlas of Poland]. Ed. Wydawnictwa Geologiczne, Warszawa.

Williams, R.H. & Fitt, B.D.L. 1999: Differentiating A and B groups of *Leptosphaeria maculans*, causal agent of stem canker (blackleg) of oilseed rape. – Plant Pathology 48: 161-75.

Xu, X.H., Hill, C.B. & Williams, P.H. 1987: Environmental conditions for the production of pseudothecia of *Leptosphaeria maculans*. – Acta Mycologica Sinica 6: 236-41.

Zhou, Y., Fitt, B.D.L., Welham, S.J., Gladders, P., Sansford, C.E. & West, J.S. 1999: Effects of severity and timing of stem canker (*Leptosphaeria maculans*) symptoms on yield of winter oilseed rape (*Brassica napus*) in the UK. – European Journal of Plant Pathology 105: 715-728.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 277-285

SimMat, a new dynamic module of Blackleg Sporacle for the prediction of pseudothecial maturation of *L. maculans/ L. biglobosa* species complex. Parameterisation and evaluation under Polish conditions

Jean-Noël Aubertot [1], Moin U. Salam [2], Art J. Diggle [3], Sylwia Dakowska [4] and Malgorzata Jedryczka [4]

[1] UMR d'Agronomie INRA/INA P-G, BP1, 78850 Thiverval-Grignon, France; [2] Department of Agriculture, Western Australia, Centre for Cropping Systems, PO Box 483, Northam, WA 6401, Australia; [3] Department of Agriculture, Western Australia, Baron-Hay Court, South Perth, WA 6151, Australia; [4] Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland

Abstract: The dynamics of pseudothecial maturation is a key process of phoma stem canker epidemics. The prediction of ascospore showers, which strongly depends on the state of pseudothecial maturity, could be useful to help decision making for fungicide application. This paper presents the structure, the parameterisation, and the evaluation of SimMat, a model that represents the dynamic of *Leptosphaeria maculans/L. biglobosa* complex species for pseudothecial maturity, in Poland. The data used in this study were collected in Poznan over four years (1998, 1999, 2000, 2001). The model uses three input variables: average harvest date, mean daily temperature, and mean daily rainfall. SimMat uses the same concept of favourable day to pseudothecial maturation as in the Blackleg Sporacle, a model developed under Australian conditions. However, the criteria used to define a favourable day for pseudothecial maturation are different and the biological variability in the requirement of a number of favourable days to maturation has been introduced differently. A day is considered to be favourable for pseudothecial maturation if the mean temperature is below a given threshold (θ_{max}) and if the accumulated rainfall over the last n_r days is greater than a threshold (r_{min}). SimMat assumes that the number of favourable days required for pseudothecial maturation follows a Gaussian distribution. SimMat has five parameters: the expected number of favourable days required for pseudothecial maturation, N_{FD}; the standard deviation of the number of favourable days required for pseudothecial maturation, σ_{FD}; θ_{max}; n_r; and r_{min}. Firstly, the model was tested using parameter values established in Australian conditions (in the Blackleg Sporacle model), with addition of σ_{FD}, which was chosen to ensure that the simulated proportion of mature pseudothecia at harvest is null. Without a calibration, as expected, the quality of prediction was poor (Root Mean Squared Error of Prediction, RMSEP = 0.44). In order to parameterise SimMat for Polish conditions, each possible combination of parameters to be adjusted was tested using cross-validation (in all 32 combinations). The best quality of prediction was obtained by adjusting just two parameters : N_{FD} and σ_{FD} (RMSEP = 0.17; bias = $-4.3\ 10^{-2}$ for cross-validation over the four years). Fitting these two parameters to the whole dataset led to a Root Mean Squared Error of 0.15, and a bias of $-4.4\ 10^{-4}$. Although this parameter estimation appears to be acceptable, the robustness of the model still has to be enhanced by increasing the size of the dataset used for parameterisation. Using this approach, the possibility of prediction of ascospore showers is discussed.

Key words: Pseudothecial maturation, *Leptosphaeria maculans*, *L. biglobosa*, stem canker, weather, prediction model

Introduction

Blackleg or stem canker of crucifers is a severe disease of oilseed rape worldwide (West *et al.,* 2001). The disease is caused predominantly by *Leptosphaeria maculans* Desm. (Ces. et de Not.), an ascomycete fungus responsible for severe cankers at root collars and stem bases of oilseed rape plants. However, in Poland and other countries of central and east Europe a related species, recently separated from '*L. maculans* species complex' and termed *L. biglobosa* (Shoemaker and Brun, 2001), is associated with profound upper stem lesions and moderate yield losses (Jedryczka *et al*., 1994, 1999b, West *et al*., 2004). In Poland, the population of *L. biglobosa* was more prevalent with respect to *L.* maculans through the 1980s until the mid-1990s (Jedryczka 1999b, Karolewski 1999), but the ratio between both species is gradually changing with *L. maculans* becoming more predominant (Karolewski *et al*., 2002; Jedryczka and Lewartowska, 2006).

Phoma stem canker is monocyclic and the main source of infection are ascospores produced in fruiting bodies (pseudothecia) on infected stubble of oilseed rape from previous seasons. Ascospore release is triggered by rainfall (Salam *et al*., 2003, Huang *et al.* 2005); the spores land on wet leaves and initiate infection. Although the pathogen can survive nearly four years in a seed (Punithalingam and Holliday, 1972), seed-borne infection is not thought to be important in epidemic initiation in comparison to air-borne infection (Wood and Barbetti, 1977). In Poland, contamination of seed samples studied by Wang (1999) was very low and varied from 0.55 % to 1.2 %. Moreover, a seed coating is a common practice and nearly 100% of commercial seeds sown are treated with a dressing.

The most severe crown cankers, originate from cotyledon and leaf lesions produced on young plants early in the growing season (West *et al.*, 2001). However, there is no direct relationship between the sowing date and the severity of phoma stem canker at the end of the crop season because of the variability in the timing of the onset of ascospore release (Aubertot *et al*., 2004). It is therefore important to know the timing of the onset of seasonal ascospore release to guide not only fungicide applications, but also to help decide on the correct sowing time of oilseed rape to reduce the risk from phoma stem canker in future seasons.

Pseudothecia must reach a complete maturity to produce and release fully developed six cell ascospores (Petrie, 1994). The process of maturation is weather dependent and there is a strong effect of rainfall. Based on studies by Toscano *et al*. (2003) it may be deduced that an influence of temperature is less crucial than wetness. This phenomenon was taken into consideration in forecasting systems. They are mainly based on rain-days, whereas high and low temperatures are used as thresholds that are borderlines for the process of pseudothecial maturity. The forecasts described by Pérès and Poisson (1999) and Salam *et al.* (2003) are based on counting days with rainfall, but temperature occurs only as upper threshold value below which pseudothecial maturation takes place. However, in cool or cold climate countries like Poland or Canada, autumn and winter temperatures drop down below 0 ^{0}C. The longevity and temperature scheme of this process decides whether pseudothecia and ascospore maturation is slowed down, stopped for a certain time or terminated. Recently, the Blackleg Sporacle model developed by Salam *et al*. (2003) has been improved by the introduction of a low temperature threshold. The model was used for predicting onset of ascospore release and a prediction value measured by a root mean squared deviation (*RMSD*) for seasonal variability in Poland was 5.6 days (Salam *et al*., 2006). The performance of the SporacleEzy model, that is simplified by the elimination of temperature and rain threshold duration parameters, was even better and the difference between observed and predicted values for Poland decreased to 5.4 days. Under Polish conditions, the minimum temperature threshold is less important for the prediction of pseudothecial maturation than for ascospore showers,

because maturation processes take place in late summer and early autumn when frosty days are not encountered.

This paper presents the structure, parameterisation, and evaluation of SimMat, a new dynamic module of Blackleg Sporacle for the prediction of pseudothecial maturation. The model represents dynamics of pseudothecia maturity for *Leptosphaeria maculans/ L. biglobosa* species complex, which is commonly found on rapeseed stubbles.

Materials and methods

Sampling procedure

For this study, data on pseudothecial maturity were collected in Poznan (central-west Poland) during four subsequent autumn seasons 1998-2001. Winter oilseed rape debris from the previous seasons experiments using two cultivars, Capitol and Lipton, which were artificially inoculated with infected stems, were kept outdoors and collected at weekly intervals from mid-August onwards. In most cases, the number of fruiting bodies studied at a given sampling date varied from 80-90, and sometimes reached 120. The minimal sample size was three stem fragments from three locations with 6 fruiting bodies each, giving a total of 54 fruiting bodies. Observations were made using a light microscope. Fruiting bodies were divided into pycnidia and pseudothecia. Maturation of pseudothecia was classified in four categories (A-D), with:

A – immature, empty pseudothecium with no asci and no ascospores;
B – pseudothecium with immature and empty asci;
C – pseudothecium with asci containing less than eight ascospores or eight immature spores with less than six cells;
D – pseudothecium with asci containing eight fully developed spores (six cells).

For each sampling date, the proportion of each category was calculated. An automatic weather station, located *Ca* one kilometre from the stubble site recorded the mean daily temperature and the daily rainfall.

Structure of SimMat

The Blackleg Sporacle model consists of two interacting modules: the first one predicts the onset of pseudothecial maturity and the second one predicts ascospore showers as a function of climatic variables when pseudothecia are mature. In Blackleg Sporacle, each day following harvest of oilseed rape is classified as either favourable for pseudothecial maturation or not. A day is declared to be favourable to pseudothecial maturation if the mean daily temperature for the preceding 10-day period is less than a threshold value, and the total rainfall for the last 7 days is greater than or equal to another threshold. A running total of days favourable to pseudothecial maturation is calculated and when this running total reaches a given value, onset of pseudothecial maturity is reached. We proposed a slightly different structure for this module (named SimMat) by simplifying the criteria used to define whether a day is favourable to maturation or not and by introducing a Gaussian distribution for the number of favourable days to maturation required. In SimMat, a day is declared to be favourable to pseudothecial maturation if the mean daily temperature is less than a threshold value (θ_{max}), and the total rainfall for the last n_r days is greater than or equal to another threshold (r_{min}). We hypothesized that, like many other biological processes (Little and Hills, 1978), the probability that a pseudothecium is mature approximately follows a bell-shaped distribution as a function of the number of accumulated favourable days to maturation. We chose to represent this distribution with a Gaussian function where N_{FD} is the expected number of favourable days required for pseudothecial maturation; and σ_{FD} is the standard deviation of the number of favourable days required for pseudothecial maturation. This structure can be expressed mathematically as follows.

Let DFM(i) be a Boolean variable equal to 1 if the day i is favourable to maturation and equal to 0 if not. Let θ be the mean daily temperature, and r be the total rainfall over the last n_r days. For the day d, the cumulated number of days favourable to maturation after harvest CDFM(d) can be expressed as:

$$CDFM(d) = 1day * \sum_{i=1}^{d} DFM(i)$$

with:

DFM(i)=1 if θ<θmax and r>r_{min} over the last n_r days
else
DFM(i)=0

The proportion of mature pseudothecia at day d, after CDFM(d) accumulated favourable days to maturation can be therefore expressed as:

$$P_{MP}(d) \cong \frac{1}{\sigma_{FD}\sqrt{2\pi}} \int_{0}^{CDFM(d)} e^{\frac{-(x-NFD)^2}{2\sigma_{FD}^2}} dx$$

SimMat is therefore a simple model that predicts an output variable (the proportion of mature pseudothecia), as a function of three input variables (the mean harvest date; the mean daily temperature and the daily rainfall from harvest to the end of the season). SimMat has only five parameters: θ_{max}; r_{min}; n_d; N_{FD}; and σ_{FD}.

Parameterisation and evaluation of SimMat

We used initial values for four parameters of SimMat using published data (Salam *et al.*, 2003): θ_{max}=22 °C; r_{min}=4.0 mm; n_d=7 days; N_{FD}=43 days. We defined the initial value of σ_{FD} to ensure that the simulated proportion of mature pseudothecia at harvest was null: σ_{FD}= 5 days.

Most of these parameters were estimated under Australian conditions, which are very different from Polish conditions, so a re-parameterisation was done using Polish data. For SimMat, one can calibrate 2^5=32 combinations of parameters (including the case where no parameter is adjusted). Several criteria can be used to evaluate a model (Wallach, 2006). We used the bias and the Root Mean Squared Error (RMSE) to characterise the agreement between measured and calculated values and the Root Mean Squared Error of Prediction (RMSEP) to characterise the predictive quality of the model. These criteria were calculated as follows.

$$Bias = \frac{1}{\sum_{i=1}^{N} N_i} \sum_{i=1}^{N} \sum_{j=1}^{Ni_i} \left(Y_{ij} - \hat{Y}_{ij}\right)$$

$$RMSE = \sqrt{\frac{1}{\sum_{i=1}^{N} N_i} \sum_{i=1}^{N} \sum_{j=1}^{Ni_i} \left(Y_{ij} - \hat{Y}_{ij}\right)^2}$$

where Y_{ij} is the j^{th} measured value for situation *i* and $\hat{Y}_{ij}$ is the corresponding value calculated by the model; N is the number of situations (4, corresponding to 4 years in this study) and N_i is the number of observations in the situation i (in this study, N_i ranges from to 2 to 7).

$$RMSEP = \sqrt{E\left\{\left[Y - f\left(X;\hat{\theta}\right)\right]\middle|\hat{\theta}\right\}^2}$$

where Y are the observations and $f\left(X;\hat{\theta}\right)$, the corresponding values calculated with the model, averaged over situations of interest; X being the explanatory variable vector and $\hat{\theta}$ the parameter vector with estimated values. The notation $|\hat{\theta}$ means that the parameter vector estimator is treated as fixed, so the expectation is not over possible values of the parameters (Wallach, 2006).

Because the dataset contains only four independent situations, we chose to perform a cross-validation. The cross-validation estimator of $RMSEP_{CV}(\hat{\theta})$ can be written as:

$$\hat{R}MSEP_{CV}\left(\hat{\theta}\right) = \sqrt{\frac{1}{\sum_{i=1}^{N} N_i}\sum_{i=1}^{N}\sum_{j=1}^{Ni_i}\left[Y_{ij} - f\left(X_{ij};\hat{\theta}_{-i}\right)\right]^2}$$

The notation $\hat{\theta}_{-i}$ indicates that the parameter values are estimated using all the data in the data set except those in situation *i*.

Each of the 32 combinations of parameters was adjusted using $RMSEP_{CV}(\hat{\theta})$ as the criterion to minimise. The mean harvesting date was considered to be July 15th for the four years included in the database. SimMat and the algorithm to estimate its parameters were programmed in C++ and compiled with C++ Builder 6.0© (Borland, 2002). The Gaussian function was calculated with the s15abc routine of NAG© libraries (CLDLL074Z; NAG©, 2002). In order to avoid problems with local minima, no optimised minimisation algorithm was used but all the possible integer values within a given range were scrutinised for the parameters to be estimated. The tested ranges were the following: $\theta_{max} \in$[10-30] °C; $r_{min} \in$[1-60] mm; $n_d \in$[1-14] days; $N_{FD} \in$[1-90] days; $\sigma_{FD} \in$[1-90] days.

Results and discussion

As expected, without a calibration, the general quality of prediction was poor ($RMSEP_{CV}$ = 0.44). The model was systematically ahead of observations (in 1998, 1999, 2000). However, the model with initial values quite satisfactorily depicted the dynamic of pseudothecial maturation in 2001 (data not shown). The best prediction quality, *i.e.* the lowest $RMSEP_{CV}$, was obtained by adjusting only two parameters: N_{FD} and σ_{FD} (Table 1). The minimum value of $RMSEP_{CV}$ for the proportion of mature pseudothecia was 0.17, and the associated bias was -4.3 10^{-2}. Fitting these two parameters to the whole dataset led to a RMSE of 0.15, and a bias of -4.4 10^{-4}. The adjusted values for these two parameters were: N_{FD}=65 d and σ_{FD}=24 d; θ_{max}, $r_{min,}$ and n_d being unchanged. One can remark that adjusting all the five parameters of SimMat led logically to the minimum value of RMSE (0.07), but that the associated $RMSEP_{CV}$ was greater than 11 combinations of 2, 3, or 4 parameters.

Over the four years of the data set, SimMat satisfactorily predicted the dynamic of pseudothecia maturation for 3 years: 1998, 1999, and 2000 (Figure 1). However, in 2001, SimMat did not succeed in predicting the rapid increase in pseudothecia maturity that had been observed. The reason why the model failed in one situation and worked well in others still requires further investigations. Nevertheless, one can remark that in this situation, the two parameters N_{FD} and σ_{FD} were adjusted with only eight observations, which is probably not sufficient to ensure the robustness of the adjustment. In this particular situation, original

Table 1. Evaluation of SimMat's predictive quality with parameters adjusted to Polish conditions and adjusted parameters for the 32 possible parameter combinations. $RMSEP_{CV}$ stands for Root Mean Squared Error of Prediction with cross-validation and characterise the predictive quality of SimMat for the proportion of mature pseudothecia. This criterion was used to rank the combination of adjusted parameters as a function of their predictive quality, the lowest $RMSEP_{CV}$ corresponding to the best quality of prediction. RMSE stands for Root Mean Squared Error and characterises the goodness of fit of SimMat. N_{AP} indicates the number of adjusted parameters. The values in the last five columns correspond to the adjusted parameters using all the observations of the database. Hyphens indicate that the initial values of the parameters were not modified. These initial values were: maximum mean daily temperature threshold θ_{max}=22 °C; minimum total rainfall within the last n_d days r_{min}=4.0 mm; n_d=7 days; expected number of days favourable required for pseudothecial maturation N_{FD}=43 days; standard deviation of the number of days favourable required for pseudothecial maturation σ_{FD}= 5 days.

$RMSEP_{CV}$	RMSE	N_{AP}	N_{FD} (d)	σ_{FD}(d)	θ_{max} (°C)	n_r (d)	R_{min} (mm)
0.1666	0.1508	2	65	24	-	-	-
0.1723	0.1434	3	54	20	-	5	-
0.1822	0.1029	3	7	-	13	-	18
0.1825	0.0764	4	11	-	14	5	11
0.1988	0.1667	2	-	18	-	4	-
0.1991	0.1391	3	61	23	-	-	5
0.2041	0.1341	3	-	16	25	-	11
0.2078	0.1479	2	-	20	-	-	11
0.2127	0.1610	2	-	26	17	-	-
0.2137	0.1865	2	14	-	-	1	-
0.2239	0.1415	3	67	19	24	-	-
0.2306	0.0728	5	11	6	14	5	11
0.2367	0.1003	4	6	4	12	-	16
0.2431	0.1530	3	-	27	16	13	-
0.2479	0.1362	3	19	-	-	3	8
0.2517	0.1339	4	56	15	25	5	-
0.2843	0.1313	3	-	14	-	5	6
0.2953	0.2458	1	67	-	-	-	-
0.3119	0.1277	4	-	14	25	5	7
0.3147	0.2359	2	-	-	16	13	-
0.3183	0.1489	3	15	-	25	1	-
0.3295	0.2412	1	-	-	-	-	9
0.3310	0.3098	1	-	52	-	-	-
0.3341	0.2435	2	39	-	16	-	-
0.3367	0.2185	2	-	-	-	5	6
0.3374	0.2278	2	-	-	19	-	9
0.3432	0.1952	2	19	-	-	-	22
0.3441	0.2585	1	-	-	17	-	-
0.3453	0.2629	1	-	-	-	4	-
0.3896	0.1155	4	25	8	-	5	11
0.4340	0.1880	3	-	-	25	5	6
0.4400	0.4400	0	-	-	-	-	-

parameters proved to be more effective than those parameterised for Polish conditions. One possible explanation may be connected with the ecological balance of the pathogen populations on rapeseed stubble and in air samples. It is not clear whether *L. maculans* and *L. biglobosa* fruiting bodies require exactly the same conditions for their maturation. The Blackleg Sporacle model was developed in Australian conditions, where only *L. maculans* ascospores have been found (Howlett *et al*., 2001). Thus, a possible prevalence of airborne spores of *L. maculans* over *L. biglobosa* in autumn 2001 could make original parameters work better that the adjusted ones. A quantitative system of analysing spore tapes in respect to presence and ratio between both species would be of great value to clarify this conjecture.

The general parameter estimation of SimMat appears to be satisfying, but its robustness still has to be enhanced by increasing the size of the dataset used for parameterisation. It is remarkable that SimMat can quite satisfactorily predict a complex biological process such as pseudothecia maturity using only two basic climatic variables. The prediction of ascospore showers will require a link to be made between SimMat and the other module of Blackleg Sporacle, which predicts ascospore ejection as soon as a rainfall event occurs. The complete adaptation of Blackleg Sporacle to Polish conditions will require a dataset that contains observations on both pseudothecial maturation of the *L. maculans/L. biglobosa* complex species and the atmospheric concentration in ascospores. Like in Australia, the prediction of ascospore showers should soon be available to help decision making to optimise time of fungicide applications.

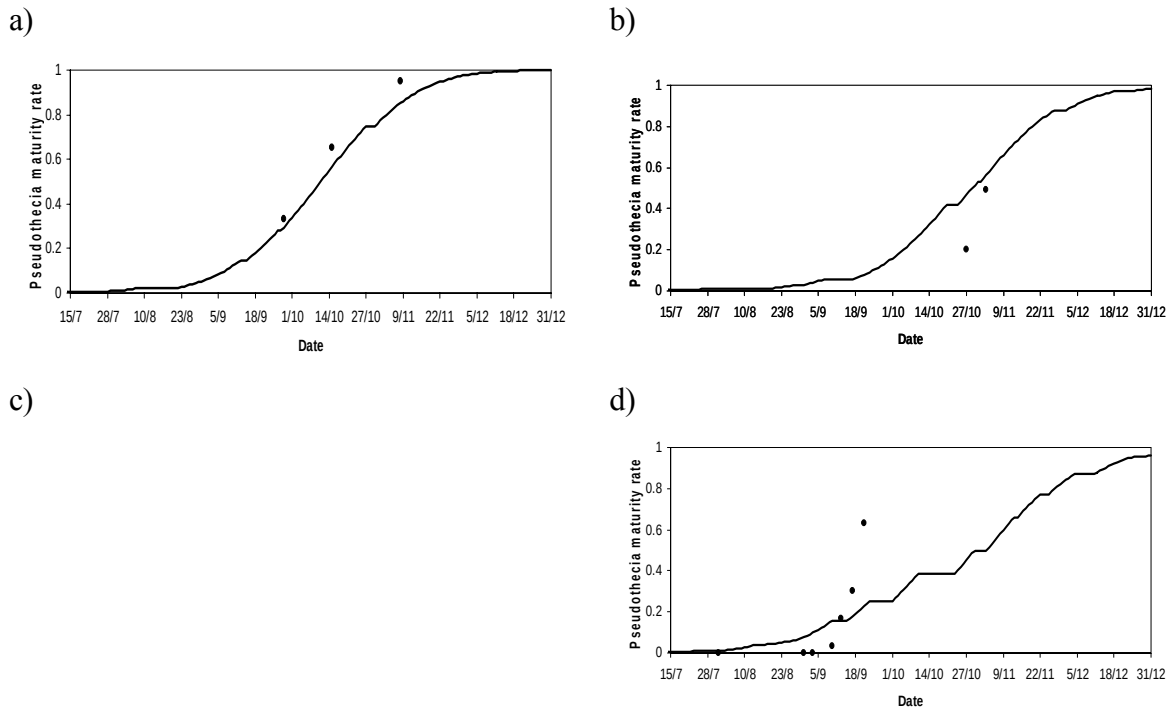


Figure 1. Evaluation of SimMat's predictive quality with N_{FD} and σ_{FD} parameters adjusted to Polish conditions (cross-validation) over four situations: Poznan in 1998 (a); 1999 (b); 2000 (c); and 2001 (d). The lines are the simulated proportion of mature pseudothecia from harvest (July 15th) to December 31st. The dots are the observations. The Root Mean Squared Error of Prediction with cross-validation is 0.17 and the bias is –0.043.

References

Aubertot, J.N., Pinochet, X. & Doré T. 2004: Analysis of the effects of sowing date and nitrogen availability during vegetative stages on phoma stem canker (*Leptosphaeria maculans*) development on two winter oilseed rape cultivars. – Crop Protection. 23 : 635-645.

Borland Software Corporation. 2002: C++ Builder 6.0 User's guide.

Howlett, B.J., Idnurm, A. & Pedras, M.S.C. 2001: *Leptosphaeria maculans* the causal agent of blackleg disease of Brassicas. – Fungal Genetic Biology. 33: 1-14.

Huang, Y.J., Fitt, B.D.L., Jedryczka, M., Dakowska, S., West, J.S., Gladders, P., Steed, J.M. & Li, Z.Q. 2005: Patterns of ascospore release in relation to phoma stem canker epidemiology in England (*Leptosphaeria maculans*) and Poland (*Leptosphaeria biglobosa*). – European Journal of Plant Pathology 111: 263-277.

Jędryczka M., Fitt B.D.L., Kachlicki P., Lewartowska E., Balesdent M.H. & Rouxel T. 1999a: Comparison between Polish and United Kingdom populations of *Leptosphaeria maculans*, cause of stem canker of oilseed rape. – Journal of Plant Pathology and Plant Protection 106 (6): 608-617.

Jędryczka, M. & Lewartowska, E. 2006: Distribution and change in *L. maculans /L. biglobosa* populations in Poland (2000-2004). – IOBC Bulletin (this volume)

Jędryczka, M., Lewartowska, E. & Frencel, I. 1994: Properties of *Phoma lingam* (Tode ex Fr.) Desm. isolates from Poland. I. Pathogenicity characterisation. – Phytopathologia Polonica 7 (XIX): 71-79.

Jędryczka, M., Rouxel, T. & Balesdent, M.H., 1999b: Rep-PCR based genomic fingerprinting of a Polish population of *Leptosphaeria maculans*. – European Journal of Plant Pathology 105: 813-823.

Karolewski, Z. 1999: Characteristics of *Leptosphaeria maculans* isolates occurring in Wielkopolska Region in 1991-1996. – Phytopathologia Polonica 18: 103-111.

Karolewski Z., Kosiada T., Hylak-Nowosad B. & Nowacka K. (2002) Changes in population structure of *Leptosphaeria maculans* in Poland. – Phytopathologia Polonica 25: 27–34.

Little, T.M. & Hills, F.J. 1978: Agricultural Experimentation: Design and Analysis. – Wiley and Sons, New York. 350 pp.

Numerical Algorithms Group. 2002: NAG C library manual. Mark 7. – NAG, Central Office, Oxford, UK.

Pérès, A., Poisson, B., Le Sourne, V. & Maisonneuve, C. 1999: *Leptosphaeria maculans*: Effect of temperature, rainfall and humidity on the formation of pseudothecia. – Proceedings of the 10th International Rapeseed Congress (http://www.regional.org.aa/au/gcirc/index.html). Canberra, Australia.

Petrie, G.A. 1994: Effects of temperature and moisture on the number, size and septation of ascospores produced by *Leptosphaeria maculans* (blackleg) on rapeseed stubble. – Canadian Plant Disease Survey 74: 141-151.

Punithalingam, E. & Holliday, P. 1972: *Leptosphaeria maculans*. – In: CMI Descriptions of Pathogenic Fungi and Bacteria. No. 331.

Salam, M.U., Fitt, B.D.L., Aubertot, J.N, Diggle, A.J., Huang Y.J., Barbetti, M.J., Gladders, P., Jedryczka, M., Khangura, R.K., Wratten, N., Fernando, W.G.D., Pinochet, X., Penaud, A. & Sivasithamparam K. 2006: Two weather-based empirical models for predicting onset of seasonal release of ascospores of *Leptosphaeria maculans* or *L.biglobosa*, causes of oilseed rape phoma stem canker in Australia, Canada, France, Poland or the UK. – Plant Pathology (submitted).

Salam, M.U., Khangura, R.K., Diggle, A.J. & Barbetti, M.J. 2003: Blackleg Sporacle: A model for predicting onset of pseudothecia maturity and seasonal ascospore showers in relation to blackleg of canola. – Phytopathology 93: 1073-1081.

Shoemaker, R.A. & Brun, H. 2001: The teleomorph of the weakly aggressive segregate of *Leptosphaeria maculans*. – Canadian Journal of Botany 79: 412-419.

Toscano-Underwood, C., Huang, Y.J., Fitt, B.D.L. & Hall A.M. 2003: Effects of temperature on maturation of pseudothecia of *Leptosphaeria maculans* and *L. biglobosa* on oilseed rape stem debris. – Plant Pathology 52: 726-36.

Wallach, D. 2006: Evaluating crop models in Working with Dynamic Crop Models. Evaluating, analysing, parameterizing and using them. – In: Wallach, D., Makowski, D. & Jones, J.W. (eds.). Working with Dynamic Models. Evaluation, Analysis, Parameterization, and Applications. Elsevier.

Wang, G. 1999: Evaluation of *Brassica napus* seed infection by *Leptosphaeria maculans / Phoma lingam.* – M.Sc Thesis, Agricultural University, Poznan, Poland.West, J.S., Jędryczka, M., Penaud, A. & Fitt, B.D.L. (2004). Comparing fungal diseases on oilseed rape in England, France and Poland. – IOBC/wprs Bulletin 27 (10): 39-44.

West J.S., Kharbanda P.D., Barbetti M.J., Fitt B.D.L. 2001: Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. – Plant Pathology 50: 10-27.

Wood, P. & Barbetti, M..J. 1977: The role of seed infection in the spread of blackleg of rape in Western Australia. – Australian Journal of Experiments in Agriculture and Animal Husbandry 17: 1040-1044.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 287-292

Prediction of the date of onset of phoma leaf spot epidemics on oilseed rape in the UK

Neal Evans[1], Andreas Baierl[1, 5], Peter Gladders[2], Beth Hall[3] and Bruce D.L. Fitt[1]
[1] *Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK;* [2] *ADAS Boxworth, Battlegate Road, Boxworth, Cambridgeshire, CB3 8NN, UK;* [3] *Syngenta Crop Protection UK Ltd, Whittlesford, Cambridge, CB2 4QT, UK;* [5] *Current address – Institute of Statistics and Decision Support Systems, University of Vienna, A-1010 Vienna, Austria*

Abstract: To reduce reliance on fungicides for control of oilseed rape diseases, Decision Support Systems (DSS) are being developed to target applications as part of an integrated disease management strategy. This paper reports on the development of an empirical model for predicting risk of severe phoma stem canker epidemics. Meteorological data are used to predict the date when incidence (% plants affected) of phoma leaf spot can be expected to reach 10%. This incidence has been suggested as an appropriate threshold to guide timing of fungicide applications against this disease to prevent pathogen spread from leaf to stem and the subsequent development of damaging stem cankers. The 10% phoma leaf spot forecast can be extended, using further meteorological and cultivar resistance rating data, to predict the date of canker onset, canker severity at harvest and subsequent yield loss.

Key words: Decision Support System, *Phoma lingam*, *Leptosphaeria maculans*, modelling, integrated control.

Introduction

Phoma stem canker (*Leptosphaeria maculans*) is the most damaging disease of winter oilseed rape in the UK (Fitt *et al*., 1997). Disease control can be achieved using fungicides but the timing of application is critical for sprays to be economically effective (Gladders *et al*., 2004). However, the risk of severe phoma stem canker epidemics differs between regions of the UK, with the most damaging phoma stem canker epidemics in eastern England (Fitt *et al*., 1996; http://cropmonitor.co.uk/commercialsurvey/csosr/riskmaps.cfm). Severity of phoma stem canker epidemics also differs between seasons and between crops within a region (Fitt *et al*., 1996; West *et al*., 2002). There is therefore a need for accurate prediction of the risk of severe epidemics to guide decisions about fungicide applications.

Materials and methods

Phoma stem canker – Crop-specific forecast (empirical model development)

A crop-specific phoma stem canker progress model, developed using meteorological data, divides epidemic progress into four stages over the season (Figure 1). Random effect models were applied to predict the onset of phoma leaf spotting. Modelling was done using data from 36 oilseed rape experiments at seven sites (Figure 2) collected between 1996 and 2001. Data consisted of weekly phoma leaf spot incidence (% plants affected) and daily rainfall and temperature. The model that gave the best fit used cumulative maximum temperature and cumulative daily rainfall between 15 July and 26 September to forecast the Julian date when 10% of oilseed rape plants can be expected to be affected with phoma leaf spotting (Figure 3).

The effect of UK resistance rating on the thermal time elapsed between onset of 10% phoma leaf spotting (in autumn) and onset of stem canker (in spring) was estimated by analysis of variance. Increase in stem canker severity with thermal time (degree-days) was analysed with random coefficient models to account for repeated assessments on the same crop. This part of the model (Figure 1, [3]) also accounts for the resistance rating of the specific cultivar being grown, with canker severity increasing faster for susceptible cultivars. Finally, the relationship between yield and stem canker severity was investigated using linear regression.

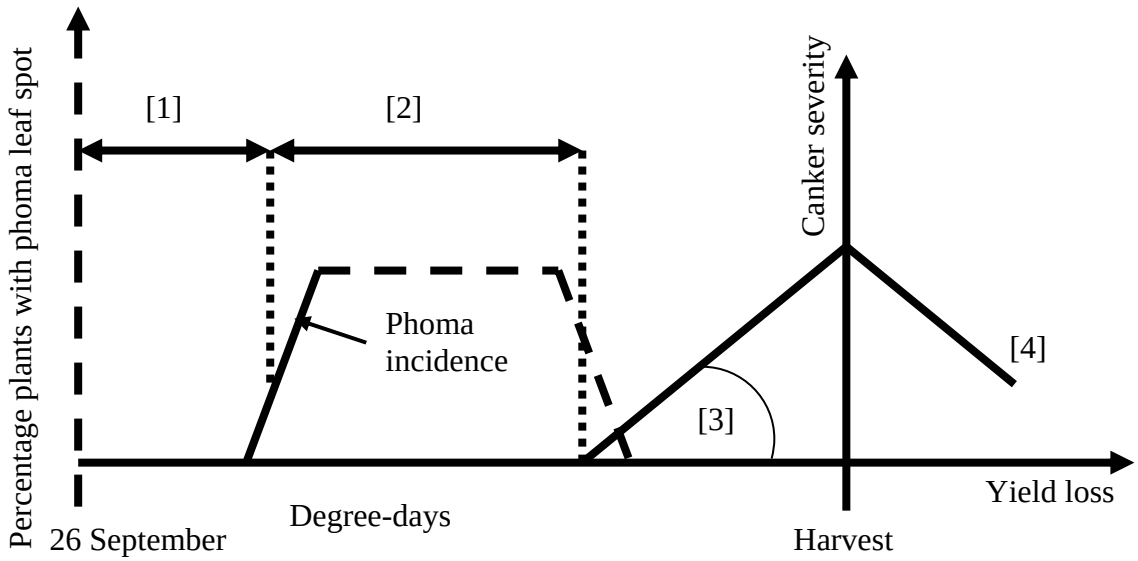


Figure 1. Schematic summary of an empirical phoma stem canker progress model. Numbers in brackets indicate stages 1 to 4: [1] Prediction of the onset of phoma leaf spotting (in autumn, 10% plants affected), [2] Time (degree-days) between onset of phoma leaf spotting and onset of stem canker development, [3] Increase in stem canker severity until harvest with time (degree-days), [4] Relationship between stem canker severity and yield loss.

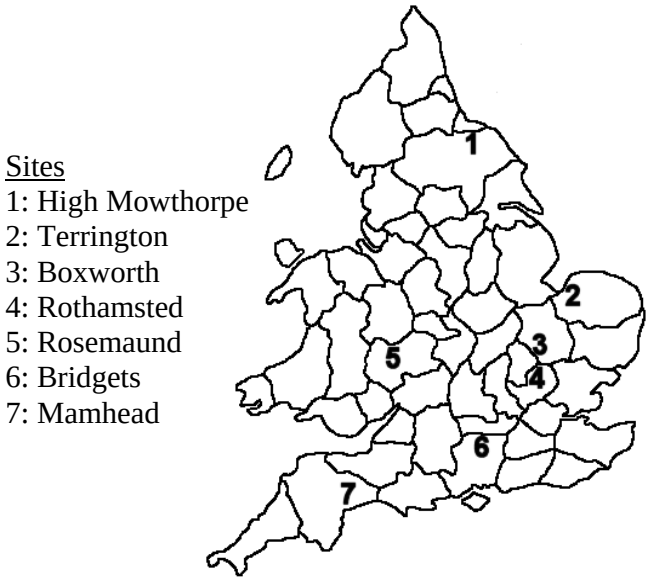


Figure 2. Map of England and Wales showing the location of winter oilseed rape experiments at which weekly incidence (% plants affected) of phoma leaf spot was measured (harvest years 1996 - 2001).

Results and discussion

Seasonal forecasts, epidemiological questions and climate change

Prediction of the date of onset of phoma leaf spotting (10% plants affected) for each season was made at the end of September, based on local temperature and rainfall data in summer (from 15 July up to 26 September), for example for autumn 2004 in the 2004/2005 growing season (Figure 4). At the end of September, scenarios for the further progress of the disease can be modelled using expected values for daily temperature in winter and spring (i.e. 30 year mean data for a given location) and information about the cultivar sown. The predicted disease progress can be updated with observed records, as new information becomes available during the season. This applies both to temperature data and to disease assessments. For example, if phoma leaf spot or stem canker symptoms are observed earlier or later than predicted, the projected prediction can be adjusted.

As only simple meteorological data are required for predictions, the model can be used to investigate epidemiological questions. For example, phoma leaf spotting occurs annually in Scottish crops, but damaging stem cankers fail to develop. Using data from Aberdeen, the model indicates the reason cankers do not develop in Scotland. Since average temperatures are lower than in England, the onset of stem canker occurs 2-3 weeks later than in the south-east of England and there is insufficient thermal time for severe stem cankers to develop before harvest (Figure 5).

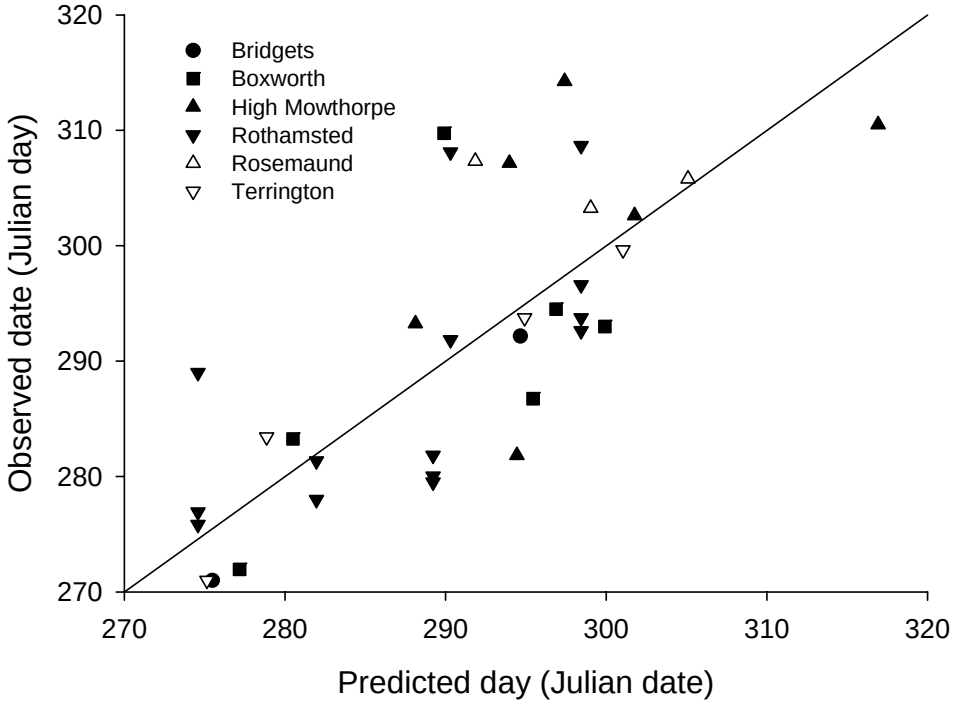


Figure 3. Relationship between observed date (Julian day) when 10% of winter oilseed rape plants were affected by phoma leaf spot in 32 experiments (at 7 sites between harvest years 1996 and 2001) and date predicted by a weather based model (408-0.27 x sum of rain – 3.6 x accumulated maximum temperature between 15 July and 26 September). Goodness of fit of predicted to observed date assessed by linear regression ($R^2 = 0.63$).

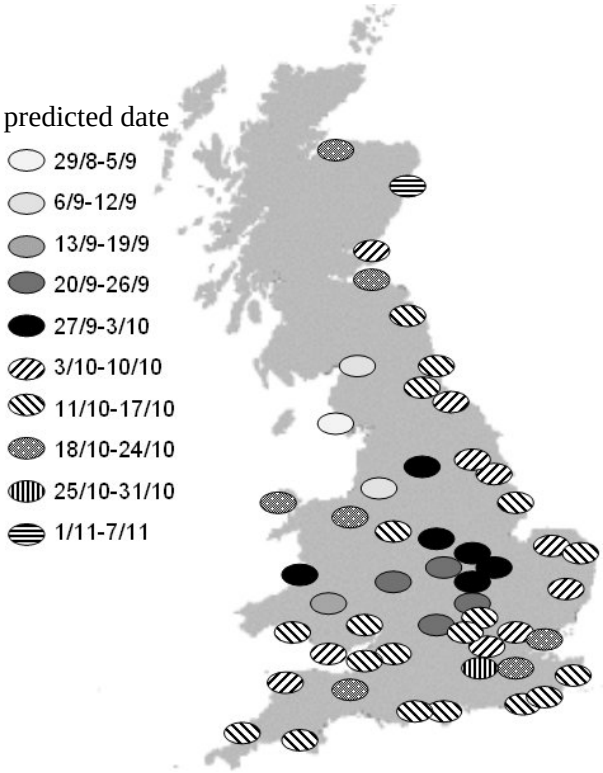


Figure 4. Predicted dates when 10% of winter oilseed rape plants were affected with phoma leaf spot at different sites across the UK for autumn 2004. Predictions made using a weather based model (408-0.27 x sum of rain – 3.6 x accumulated maximum temperature between 15 July and 26 September) using data from UK Meteorological Office stations.

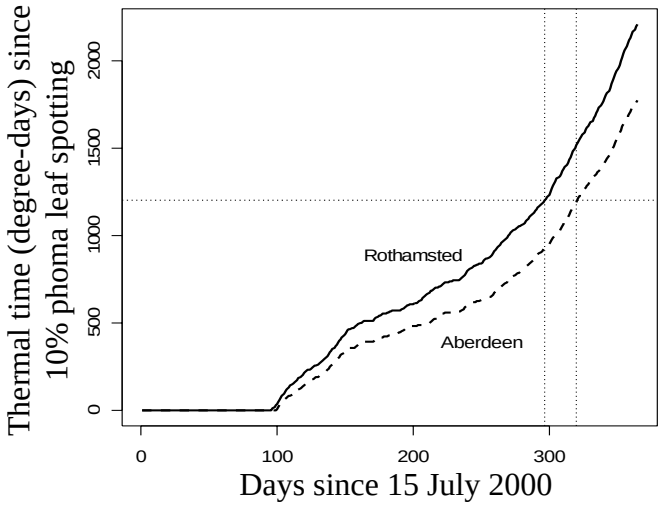


Figure 5. Predicted phoma stem canker development at Rothamsted, England and Abderdeen, Scotland based on meteorological data for 2000/2001. The dotted line (1200 degree-days) indicates the predicted date for onset of canker development in spring. The model suggests that, at Aberdeen, canker onset occurs 2-3 weeks later than in south east England (e.g. Rothamsted) leaving insufficient time for damaging canker development before harvest.

Manipulation of meteorological data to simulate scenarios of different climatic conditions that may occur due to climate change also allows us to investigate the possible effect that climate change may have on the severity of phoma stem canker epidemics. Most climate change models suggest a 2-3°C increase in the UK mean temperatures in the short-term (e.g. by 2050) with further increases by as much as 4°C by 2080 (Beniston, 2004). Climate change scenarios suggest that even a 1.5°C increase in average temperature would result in an increase in severity of the phoma stem canker epidemic (Figure 6). In an average season at Rothamsted (e.g. 2004 meteorological data, Figure 6), canker onset was predicted to be 290 days after 15 July which is 1 May. Early canker symptoms were first observed at Rothamsted in early May during 2005 and developed to a level of moderate canker severity by harvest. However, with a 1.5°C increase in temperature, the model suggests that canker onset would be shifted forward with onset predicted on day 210 after 15 July, which is 10 February, 18 weeks prior to onset under current climatic conditions (Figure 6).With such an early onset date and with higher average temperatures aiding subsequent canker development within stem tissues, the model suggests that phoma stem canker epidemics may become extreme if conditions develop as current climate change models predict.

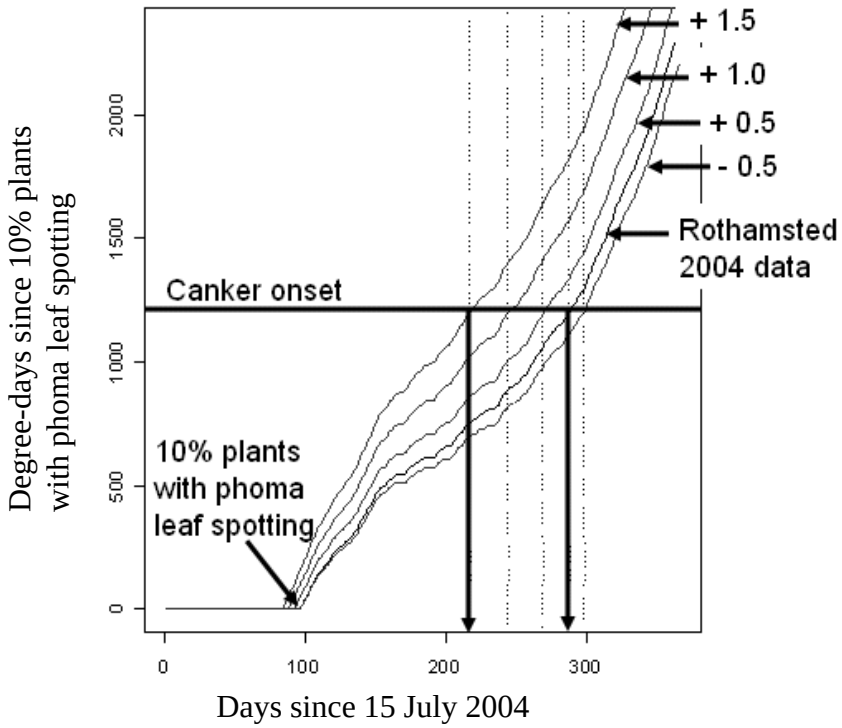


Figure 6. Predicted date in spring 2005 of onset of phoma stem canker at Rothamsted made in autumn 2004 and predictions produced for climate change scenarios of a -0.5°C, +0.5°C, +1°C and +1.5°C change in average temperature during the growing season showing an increase in the severity of the phoma stem canker epidemic with increasing temperature (as onset of canker development is earlier).

Acknowledgements

We thank the HGCA, Perry Foundation, Syngenta and DuPont for funding and Defra (UK Department for Environment Food and Rural Affairs) LINK for sponsorship. We also thank ADAS and Rothamsted Research staff for help collecting data at experimental sites. Rothamsted

Research receives funding from the UK Biotechnology and Biological Sciences Research Council.

References

Beniston, M. 2004: The 2003 heat wave in Europe: A shape of things to come? An analysis based on Swiss climatological data and model simulations. – Geophysical Research Letters 31, L02202, doi:10.1029/2003GL018857.

Fitt, B.D.L, Gladders, P., Turner, J.A., Sutherland, K.G. & Welham, S.J. 1996: Predicting risk of severe light leaf spot (*Pyrenopeziza brassicae*) on winter oilseed rape in the UK. – Proceedings British Crop Protection Conference - Pests and Diseases, 239-244.

Fitt, B.D.L, Gladders, P., Turner, J.A., Sutherland, K.G., Welham, S.J. & Davies, J.M.L. 1997. Prospects for developing a forecasting scheme to optimise use of fungicides for disease control on winter oilseed rape in the UK. – Aspects of Applied Biology 48 (Optimising Pesticide Applications): 135-142.

Gladders, P., Jewell, K. & McDonough, S. 2004: The contribution of cultivar resistance and fungicides to disease control in winter oilseed rape in England. – IOBC/wprs Bulletin 27 (6): 51-56.

West, J.S., Fitt, B.D.L., Leech, P.K., Biddulph, J.E., Huang, Y.J. & Balesdent, M.H. 2002: Effects of timing of *Leptosphaeria maculans* ascospore release and fungicide regime on phoma leaf spot and phoma stem canker development on winter oilseed rape (*Brassica napus*) in southern England. – Plant Pathology 51: 454-463.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 293-300

Studies on the contribution of cultivar resistance to the management of stem canker (*Leptosphaeria maculans*) in Europe

Peter Gladders[1], Hortense Brun[2], Xavier Pinochet[3], Malgorzata Jedryczka[4], Ingrid Happstadius[5] and Neal Evans[6]
[1] ADAS Boxworth, Boxworth, Cambridge CB3 8NN; [2] UMR BIO3P, Domaine de la Motte, Le Rheu Cedex, France; [3] CETIOM, Thiverval-Grignon, France; [4] Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland; [5]Svalöf Weibull AB, 268 81 Svalöv, Sweden; [6] Rothamsted Research, Harpenden, Herts AL5 2JQ,, UK

Abstract: The development of phoma leaf spot and stem canker was investigated on a range of 7-10 cultivars at 9 sites in France, Germany, Poland, Sweden and UK in 2002/2003 and 2003/2004. The cultivars selected differed in their major gene and quantitative resistance to stem canker and included near isogenic lines of two cultivars without (Darmor, and Eurol) or with (Darmor MX, Eurol MX) major resistance gene *Rlm6* and their parent cultivars. The lines with *Rlm6* gave very effective control of phoma leaf spot and stem canker caused by *Leptosphaeria maculans*. At some sites, there was a significant increase in the incidence of *Leptosphaeria biglobosa* on leaves and stems on the *Rlm6* lines. There were differences in the severity of stem canker between the other cultivars, which reflected the differences in their quantitative resistance. Populations of *L. maculans* in Europe are known to have a high frequency of virulence to overcome resistance genes *Rlm1-4* and *Rlm9* and therefore quantitative resistance makes an important contribution to stem canker control. Strategies are required to ensure that the durability of resistance to stem canker is improved.

Key words: Phoma leaf spot, major gene, quantitative resistance, *Leptosphaeria biglobosa*

Introduction

Phoma stem canker (*Leptosphaeria maculans*) is the most important disease of winter oilseed rape in Europe (West et al., 2001). In regions where the crop is grown intensively, there is a high risk of yield loss and control measures are required routinely. It is a monocyclic disease started by spread of air-borne ascospores from the stubble of the previous season's crops to new crops in autumn and winter. Measures to reduce the spread of ascospore inoculum from the stubbles include ploughing or incorporation of crop residues after harvest and the planting of new crops at a distance of several hundred metres from previous crops. These measures are not usually sufficient to prevent some yield loss and therefore cultivars with good resistance to stem canker are required to ensure that production is economically viable. Resistance to stem canker is based on major genes operating in combination with polygenic or quantitative resistance. Single major gene resistance has been overcome within a few seasons of commercial use (Rouxel et al., 2003) and new strategies to improve the durability of cultivar resistance are required. More durable cultivar resistance would reduce the need for fungicide treatments, which are cost-effective on susceptible and moderately resistant cultivars (Gladders et al., 2004). This paper presents the results from the first two seasons of field experiments in the EU-funded SECURE project to evaluate the resistance of a range of winter oilseed rape cultivars to stem canker in different countries in Europe.

Material and methods

Replicated field experiments were sown in France, Germany, Poland, Sweden and the UK (Table 1) in late August or early September in 2002 and 2003 using seed of 7-10 winter oilseed rape cultivars/lines provided by INRA, Rennes, France. Standard agronomic treatments with fertiliser and pesticides were used according to local practice but no fungicide sprays were applied. The winter oilseed rape included near isogenic lines with (DarmorMX and EurolMX) or without the stem canker resistance gene *Rlm6* in a high (Darmor),or low (Eurol) quantitative resistance background (respectively), which were compared with selected commercial cultivars (Table 2). Seed rates were adjusted at each site to produce 50-60 plants/m^2. Natural phoma stem canker epidemics were allowed to develop at each site, but stubble inoculum was added after sowing at Rennes in autumn 2002. Disease assessments were done at 4-8 week intervals from early autumn onwards on samples of 10 plants per plot. The incidence (% plants affected) and severity (% leaf area affected) of each foliar disease was recorded. Stem diseases were recorded on 10 plants per plot before the end of flowering and on 25 plants per plot pre-harvest. Stem disease incidence and severity was recorded using a 0-5 index for stem canker (0 – no disease, 5- plant dead) and converted to a 0-100 disease index; in France, Sweden and Germany a more detailed 0-9 index (9 – plant broken or dead) was used for stem canker severity and also converted to a 0-100 index. Selected leaf and stem data are presented in this paper, together with yield data (adjusted to 90% dry matter) where this was collected.

Table 1. Sites for winter oilseed rape cultivar experiments in 2003 and 2004 harvest years.

Harvest year	Location	Date sown Date harvested	Onset of phoma leaf spot
2003	ADAS Boxworth, Cambridge, UK	6 September 2002 harvest 19 July 2003	5 November 2002
2003	Grignon, France	2 September 2002	28 October 2002
2003	Rennes, France	3 September 2002	7 November 2002
2003	Teendorf, Germany	27 August 2002, harvest 14 July 2003	16 October 2002
2004	ADAS Boxworth, Cambridge, UK	3 September 2003	8 December 2003 (1 leaf at end Oct)
2004	Flugkroken, Sweden	26 August 2003	15 November 2003
2004	Teendorf, Germany	27 August 2003	11 November 2003
2004	Cerekwice, W. Poland	4-5 September 2003	12 November 2003
2004	Grabow, E. Poland	5-6 September 2003	15 November 2003

Results and discussion

In autumn 2002, phoma leaf spot developed rapidly by late autumn and a significantly lower incidence and severity of leaf spotting was observed on the MX lines at all sites. There were few consistent differences between the other cultivars. Shogun was the most severely affected cultivar in 2002/03 although differences were not significant at Grignon. In 2003/04, Shogun had more severely affected leaves only at Teendorf. In autumn 2003, the phoma leaf spot epidemic was later than usual because of the dry autumn; this also delayed germination of oilseed rape seed at some sites. There was again a significantly lower incidence of phoma leaf

spot on the MX lines at all sites. Small dark leaf spots attributable to *Leptosphaeria biglobosa* (previously referred to as B-type phoma lesions) were present at many of the sites. At Boxworth, there was a much higher incidence of spotting by *L. biglobosa* on EurolMX and DarmorMX (20-40% plants affected) in January and February 2003 than on all other cultivars (0-5% plants affected).

Table 2. Resistance to stem canker in cultivars used in European experiments.

Reference code	Cultivar	Major gene resistance (Rlm)	Quantitative resistance
1	Apex	9	Intermediate
2	Darmor	9	High
3	DarmorMX	6, 9	High
4	Escort	1, 3	Intermediate
5	Eurol	2, 3	Low
6	EurolMX	2, 3, 6	Low
7	Falcon	4	Intermediate
8	Samourai	2	Low
9	Shogun	?	Very low
10	Capitol	1, 3	Low

Table 3. Phoma leaf spot incidence (% plants affected) and severity (% leaf area affected) in autumn/winter 2002/03.

Cultivar	Boxworth 4 Dec 02		Grignon 14 Nov 02		Rennes 25 Nov 02	Teendorf 15 Nov 02	
	% plants	% area	% plants	% area	% plants	% plants	% area
Apex	92.5	1.42	72.5	4.98	-	55.0	0.79
Darmor	95.0	1.24	82.5	5.15	98.3	42.5	0.48
Darmor-MX	2.5	0.01	5.0	0.05	4.2	0.0	0.00
Escort	85.0	0.80	77.5	3.63	-	25.0	0.19
Eurol	90.0	1.27	82.5	4.30	95.8	37.5	0.29
Eurol-MX	12.5	0.11	15.0	0.15	7.5	0.0	0.00
Falcon	75.0	0.67	75.0	2.50	-	40.0	0.33
Samourai	95.0	1.42	77.5	5.28	-	45.0	0.55
Shogun	97.5	2.26	75.0	5.30	-	67.5	1.19
Capitol	-	-	67.5	2.48	97.5	-	-
Sed	9.10	0.258	8.50	1.39	5.12	7.49	0.291
F pr	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.008
df	24	24	24	24	12	24	24

Table 4. Phoma leaf spot incidence (% plants affected) and severity (% leaf area affected) in autumn/winter 2003/04.

Cultivar	Boxworth *22 Feb 04*		Teendorf *18 Dec 03*		Flugkroken *15 Dec 03*		Cerekwica *15 April 04*		Grabow *29 April 04*	
	% plants	% area	% plants	% area	% plants	% area	% plants	% area	% plants	% area
Apex	92.5	4.46	5	0.04	75	1.63	-	-	-	-
Darmor	100	5.14	0.7	0.01	45	0.65	46.7	0.65	11.8	0.14
Darmor-MX	5	0.02	2.5	0.01	10	0.1	14	0.16	8	0.08
Escort	87.5	3.98	12.5	0.1	62.5	1.25	-	-	-	-
Eurol	100	3.84	2.5	0.01	62.5	1	28.7	0.35	37.8	0.43
Eurol-MX	7.5	0.09	0	0	20	0.28	2.7	0.03	2.2	0.02
Falcon	95	4.85	10	0.09	45	0.58	16	0.2	15.7	0.17
Samourai	97.5	4.57	10	0.06	50	0.85	31	0.36	50.8	0.69
Shogun	90	3.18	30	0.28	72.5	1.45	44.3	0.57	26.2	0.31
Capitol	-	-	-	-	-	-	23.7	0.27	-	-
Sed	5.53	1.368	6.92	0.05	14.23	0.344	6.36	0.116	7.12	0.09
F pr	<0.001	0.003	0.002	<0.001	0.001	0.002	<0.001	0.002	<0.001	<0.001
df	24	24	23	23	24	24	14	14	18	18

Stem canker symptoms were observed at all sites pre-harvest and were moderately severe or severe in 2003 (Table 5), but mainly moderately severe in 2004 (Table 6). The MX lines developed significantly less stem canker than other cultivars but at some sites there was still considerable stem canker development on MX lines. This was also observed in Poland where upper stem lesions were common despite low levels of *L. maculans* on leaves. The other cultivars could often be separated statistically into susceptible (e.g. Shogun, Samourai and Eurol) and moderately susceptible (e.g. Darmor, Escort, Apex, Falcon) groups, though this was less apparent in France. Phoma stem lesions on the lower stem developed at some sites and there were some significant differences between cultivars. EurolMX had particularly severely affected stems at Teendorf in 2003. Eurol and EurolMX had more severe stem lesions than Darmor and DarmorMX at Boxworth and Teendorf but not in Poland (Table 7).

Table 5. Effect of winter oilseed rape cultivar on stem canker incidence (% plants affected) and severity (0 – 100 index) in 2003.

Cultivar	Boxworth 24 June 03		Grignon 18 June 03	Rennes 09 June 03		Teendorf 24 June 03	
	Canker % plants	Canker index	Canker Index	Canker % plants	Canker index	Canker %plants	Canker index
Apex	97.0	54.6	56.4	-	-	52.5	29.3
Darmor	93.0	44.8	48.0	96.9	40.7	50.5	29.6
Darmor-MX	10.0	3.8	32.4	15.5	4.3	18.2	11.7
Escort	81.0	46.8	61.6	-	-	41.2	21.8
Eurol	99.0	69.0	54.0	99.6	84.8	58.5	36.6
Eurol-MX	7.0	3.8	37.2	21.3	5.0	33.8	22.4
Falcon	94.0	70.4	59.1	98.8	73.1	60.0	32.9
Samourai	99.0	81.6	58.4	100.0	88.9	67.8	40.9
Shogun	100.0	85.8	58.9	97.8	66.6	74.5	42.9
Capitol	-	-	-	98.8	76.8	-	-
Sed	4.51	6.69	9.89	5.52	4.89	7.20	4.47
F pr	<0.001 skew	<0.001	0.050	<0.001	<0.001	<0.001	<0.001
Df	24	24	24	21	21	24	24

Yield data were available only from two experiments and Shogun produced the lowest yield in both. Most cultivars produced >1.0 t/ha higher yield than Shogun (Table 7). DarmorMX had a significantly higher yield than Darmor at Boxworth but not at Teendorf. EurolMX produced a low yield at Teendorf and was similar to Eurol at Boxworth.

Phoma leaf spot epidemics developed at all sites and differed between years because early autumn rainfall was lower in autumn 2003 than autumn 2002 and this delayed maturation of ascospores. Colder winters in Poland, Germany and Sweden limited the development of phoma in winter compared with the UK and France. The risk of severe stem canker is greatest where phoma leaf spot appears in early autumn and reaches a high incidence on small plants. Severe stem cankers and phoma stem lesions were evident in 2003 and low yields were recorded in susceptible cultivars. There was a poor relationship between the yield of different cultivars and stem canker severity. Many factors influence the

performance of cultivars and further experiments with fungicide treatments to control stem canker are needed to define disease-yield loss relationships for individual cultivars.

Table 6. Effect of cultivar on stem canker incidence (% plants affected) and severity (0 – 100 index), 2004.

Cultivar	Boxworth 29 June 04		Teendorf 29 June 04		Flugkroken 24 June 04	
	Canker % plants	Canker index	Canker % plants	Canker index	Canker % plants	Canker index
Apex	92.0	57.7	19.9	12.3	54.5	30.0
Darmor	93.3	39.7	30.2	12.1	10.8	10.0
Darmor-MX	26.7	8.0	2.0	3.3	4.0	3.6
Escort	77.3	44.0	28.2	14.8	40.0	21.2
Eurol	96.0	68.0	43.0	23.2	54.5	29.7
Eurol-MX	30.7	14.7	27.1	16.5	4.2	4.2
Falcon	77.3	40.3	26.3	14.9	22.5	12.4
Samourai	97.3	75.3	50.0	26.9	71.0	39.7
Shogun	92.0	73.7	64.3	39.0	57.8	35.0
Sed	9.27	8.28	7.73	4.15	7.18	3.89
F pr	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
df	24	16	23	23	24	24

There were some differences in stem canker severity between cultivars and the benefits of the *Rlm6* resistance gene were clearly demonstrated. Moderate resistance was shown by several recent commercial cultivars. Their major gene resistance is likely to have had little effect on stem canker development as populations of *L. maculans* have been characterised at these sites and most isolates could overcome *Rlm1, Rlm 2, Rlm3*, *Rlm4* and *Rlm9* (Stachowiak et al., 2006). *Rlm6* has not been used commercially and the corresponding virulent allele is only present at low frequency or absent from most populations. It is possible that some virulent (*avrLm6*) isolates were present in the population at Grignon where stem canker control was less effective on MX lines than at other sites. Thus, the resistance of these cultivars is believed to be dependent on their quantitative background resistance. This was clearly shown in comparisons of stem canker index for Eurol (susceptible background) and Darmor (resistant background), with smaller effects when quantitative resistance was less (e.g. Shogun). There appear to be quantitative resistance factors contributing to control of phoma stem lesions as Eurol/EurolMX had more severe lesions than Darmor/DarmorMX.

On the MX lines, it was confirmed at some sites that there was a greater incidence of leaf spots caused by *L. biglobosa* than on other cultivars. The prevalence of both stem cankers and stem lesions on MX lines was also associated with *L. biglobosa*. This was confimed both by culturing on agar and by PCR diagnostics and shows that both *Leptosphaeria* species can colonise leaves, stems and the stem base of the plant (Eckert et al., 2004). There is a potential for yield loss from *L. biglobosa* and plant breeders will need to ensure that cultivars susceptible to *L. biglobosa* are not released inadvertantly by concentrating exclusively on *L. maculans*. The presence of severe phoma stem lesions (in addition to crown cankers) is also

of concern and selection for resistance to both *Leptosphaeria* spp. in stems should be included in breeding strategies.

Table 7. Effect of cultivar on phoma stem lesion incidence (% plants affected) and severity (0 – 100 index), 2003 and 2004.

.

Cultivar	Boxworth *24 June 03*		Teendorf *24 June 03*		Boxworth *29 June 04*		Cerekwica *14 July 04*		Grabow *05 July 04*	
	Phoma stem lesion		Phoma stem lesion		Phoma stem lesion		Phoma stem lesion		Phoma stem lesion	
	%plants	index	%plants	index	%plants	index	%plants	index	%plants	index
Apex	74.00	29.00	58.20	27.20	94.70	25.30	-	-	-	-
Darmor	66.00	25.40	24.70	13.40	97.30	28.70	76.30	40.80	38.20	5.30
Darmor-MX	53.00	21.80	26.50	15.10	94.70	27.30	75.00	42.70	31.20	6.60
Escort	67.00	25.40	52.50	26.30	94.70	17.30	-	-	-	-
Eurol	73.00	40.20	63.20	31.20	93.30	47.00	53.00	26.50	39.80	4.70
Eurol-MX	57.00	33.60	96.00	59.90	94.70	41.70	47.00	25.30	33.20	4.90
Falcon	65.00	32.40	54.00	27.80	96.00	31.30	65.70	30.30	37.80	6.00
Samourai	63.00	34.60	54.80	27.80	94.70	40.30	37.30	14.90	37.50	8.80
Shogun	52.00	22.60	21.70	14.80	90.70	27.30	61.30	30.70	34.50	5.80
Capitol	-	-	-	-	-	-	59.40	30.20	-	-
Sed (24df)	6.74	4.38	9.00	4.02	5.22	7.46	6.99	3.57	7.92	3.12
F test	0.025	0.004	<0.001	<0.001	ns	0.026	<0.001	<0.001	ns	ns
df	74.00	29.00	58.20	27.20	94.70	25.30	-	-	-	-

Table 8. Yield and yield response of cultivars at Boxworth and Teendorf 2003.

Cultivar	Boxworth 2003		Teendorf 2003	
	Yield (t/ha)	Yield response (t/ha) (over Shogun)	Yield (t/ha)	Yield response (t/ha) (over Shogun)
Apex	2.63	1.55	3.42	1.00
Darmor	2.33	1.25	3.93	1.51
Darmor-MX	2.84	1.76	4.14	1.72
Escort	2.47	1.39	4.29	1.87
Eurol	2.19	1.11	4.11	1.69
Eurol-MX	2.34	1.26	2.61	0.19
Falcon	1.61	0.53	4.19	1.77
Samourai	1.91	0.93	3.18	0.76
Shogun	1.08	0.00	2.42	0.00
Sed (24df)	0.225		0.289	
CV (%)	14.8		11.2	
F test	<0.001		<0.001	

Acknowledgements

This work was funded by the European Union through the SECURE project (QLK5-CT-2002-01813) and in the UK with additional funding from the Department for Environment, Food and Rural Affairs and Biotechnology and Biological Sciences Research Council.

References

Eckert, M., Fitt, B. & Selley, A. 2004: *Leptosphaeria maculans*, *L. biglobosa* and fungicides, preliminary results from *in vitro* and winter oilseed rape experiments. IOBC/wprs Bulletin 27 (6): 157-164.

Gladders, P., Jewell, K. & McDonough, S. 2004: The contribution of cultivar resistance and fungicides to disease control in winter oilseed rape in England. IOBC/wprs Bulletin 27 (6): 51-56.

Rouxel, T., Penaud, A., Pinochet, X., Brun, H., Gout, L., Delourme, R., Schmit, J. & Balesdent, M-H. 2003: A ten-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the *Rlm1* resistance gene of oilseed rape. – European Journal of Plant Pathology 109: 871-881.

Stachowiak, A., Olechnowicz, J., Jędryczka, M., Rouxel, T., Balesdent, M-H., Happstadius, I., Latunde-Dada, A., Gladders, P. & Evans, N. 2006: Frequency of avirulence genes in field populations of *Leptosphaeria maculans* in Europe. – European Journal of Plant Pathology 114: 67-75.

West, J.S., Kharbanda, P.D., Barbetti, M.J. & Fitt, B.D.L. 2001: Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. – Plant Pathology 50: 10-27.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 301-306

Controlling the deployment of a new specific resistance Rlm7 to *Leptosphaeria maculans* in a small production area, in the Centre of France

Annette Penaud[1], Emmanuelle Mestries[1], Gilles Sauzet[2] and Xavier Pinochet[1]
[1] *CETIOM, BP4, 78850 Thiverval-Grignon, France*
[2] *CETIOM, 3 rue F. Pyat, 18400 Saint-Florent sur Cher, France*

Abstract: Recently new oilseed rape cultivars resistant to stem canker have been made commercially available. Their efficiency against the pathogen *Leptosphaeria maculans* is mainly due to a new specific resistance gene Rlm7. However, such varieties could rapidly be exposed to a risk of resistance break down. To prevent such an event, an extension approach of deployment of resistance cultivars has been carried out in a small area (15 km²) in the Central region of France where stem canker pressure used to be high. This approach of durable management of genetics resistance has involved about twenty oilseed rape fields with a cropping ratio of Rlm7 resistant cultivars of about 5%. Observations are focused on technical practices, agronomic diagnosis, foliar and stem symptoms and population structure of *L. maculans*. A particular attention is paid to the emergence of leaf spot symptoms on the Rlm7 cultivars. These observations will be associated with extension recommendations for controlling stem canker to the farmers and local advisors. Evolution in time will be followed over four years. This paper presents results of the initial year.

Key words: Phoma leaf spot, major gene, quantitative resistance, *Leptosphaeria maculans*

Introduction

Stem canker (*Leptosphaeria maculans*) is the most important disease of winter oilseed rape in France and in Europe. Plant genetic resistance is the main control measure used to control the disease. Quantitative resistance and major genes are generally combined in commercial genotypes. Single major gene resistance have been overcome within a few years of commercial use (Rouxel et al., 2003). In such cases the behaviour of a given cultivar could move rapidly from resistant to susceptible, which is unacceptable for farmers. In recent years, several new major genes have been identified and tested. Field experiments have shown that such major gene could be quickly overcome (Brun et al 2000). Nevertheless, new commercial lines and hybrids having Rlm7, an efficient new major gene, are being released on to the market to begin their commercial development. After the break down of Rlm1, much applied research was done to investigate deployment measures and bio-vigilance in order to avoid further breakdowns of resistance. Recently, CETIOM has promoted an “alternance strategy” in the use of genotypes based on knowledge of both quantitative and major resistance in different cultivars (Pinochet et al 2004). Incorporation of crop residues after harvest to reduce the spread of ascospore inoculum from infected stubble is the second main strategy for control being demonstrated by CETIOM to farmers. Nevertheless classical communication tools are not sufficient. There is a need to increase credibility and to demonstrate the importance of the recommendations at the production level. This paper describes a bio-vigilance approach that CETIOM has started to develop in the Center of France in a small production area where the risk of Phoma leaf spot is relatively high and where new Rlm7 varieties are being introduced. There are three main objectives: (i) to follow in time what is happening in an area of several

km² where Blackleg risk is very high: epidemic scenario, symptoms, production impact (ii) To produce demonstration sites to support extension and communication activities. (iii) To produce data to feed into modelling activities.

Material and methods

The working area is located between St Florent sur Cher and Issoudun, incorporating 3 villages about 250 kilometres south of Paris. The production area is a square of 15 x 15 km. In this area, 20 Fields were regularly assessed during the cropping season at emergence, before winter during the second half of November, at the end of winter, during flowering, and at the beginning of ripening. The 20 fields represent 420 ha of WOSR during the 2004-05 season. Fields and the sampling areas within fields were located using a GPS system. Agronomic practices were obtained by questioning farmers. Sampling methods and agronomic observations taken were those recommended in CETIOM's WOSR experimentation guide (i.e. plant density, plant fresh weight, flowering type, height, yield components, grain yield). The main genotypes grown were: ES Astrid (Rlm9) 9 fields, Aviso (Rlm9) 5 fields, Kosto (Rlm9) 4 fields, Campala (Rlm9) 1 field, and Roxet (Rlm7) 2 fields. Ascospore trapping was done at Rosière, on the east side of the working area, using a dynamic spore trap from the 1st September to the 30th November 2004, with daily counts of ascospores being made under the microscope (Peres et al 1999, West et al 1999). Leaf spots were recorded in autumn from the 15 - 24 November in the different fields, on 4 samplings of 25 plants. The final disease index G2 was determined from the observation of 8 samplings of 5 plants (Pierre and Regnault 1982, Aubertot et al 2004). 20 leaves per field, with at least one leaf spot per leaf, were collected in November for pathogen isolation. Single-pycnidium isolates were collected from one lesion per infected leaf (West et al 2002). The virulence of the isolates will be characterised using the procedure described by Balesdent et al 2006.

Results and discussion

Fields were sown from 26 August - 4 September 2004. Plant densities before winter ranged from 15 to 111 plants m^{-2}, but averaged around 40 plant m^{-2}. Rain during October, and a dry November resulted in regular vegetative growth that was not excessive. Winter was dry and relatively cold. Flowering was late during the second half of April, after a cold period during March. The number of pods produced was rather low, but fertility was good producing a high number of seeds per pod. The thousand seed weight was intermediate at around 4 grams (data not shown). Grain yields were good for that particular area of OSR production.

Autumn 2004 was rather dry. Only low ascospore releases occurred during end of September and end of October. Main peaks were observed in late autumn. Nevertheless leaf spots occurred at the beginning of November.

The number of leaf spots observed was low, except for 4 fields (see figure 2). For each genotype there was a quite large variability between fields. The agronomic diagnosis performed on each of these fields suggested that there was a correlation between plant growth and leaf spots, and soil tillage techniques, especially when rotative superficial tillage methods were used. When low tillage techniques were used, plant growth was lower, and symptoms were less numerous.

Table 1. Fields locations and description of the working area (GPS: geographic positioning system; FW: fresh weight; Flow.: Flowering; Nb : number; qx : quintal; ha : hectare)

Filed name	GPS Field localisation		Sowing		Plants	Before Winter	Flow. time	G2 Index	Nb of pods	grain yield
	Lattitude	Longitude	date	genotype	/m2	FW g/m2	Hight cm		/m2	qx/ha
La bruère	46,94519233	2,240383008	26/08/05	Astrid	39	950	135	1	5460	38
Ballay	46,90770576	2,178219062	28/08/05	Astrid	37	2595	142	2,58	5220	37
Le petit bois	46,92087614	2,142990804	28/08/05	Astrid	44	605	122	4	6048	30
La Vigne	46,96061525	2,18643805	01/09/05	Astrid	42	585	132	2,25	6567	37
Champ des p	46,96037342	2,145082036	03/09/05	Astrid	52	655	130	1,8	5096	34
La Bataillerie	46,95660068	2,148511668	03/09/05	Astrid	38	555	125	0,75	5200	34
La Boutanderie	46,90497632	2,047618367	01/09/05	Astrid	32	810	140	1,15	6403	43
Maison rouge	46,97240775	2,067692722	30/08/05	Roxet	46	970	160	3,3	7000	37
Les Rosiers	46,96414274	2,072661984	29/08/05	Roxet	23	1005	172	1,3	6930	38
Sérille	46,97397249	2,154102359	01/09/05	Astrid	41	985	142	2	6601	38
Ballay	46,91121204	2,176567711	28/08/05	Aviso	36	1930	130	2,72	6370	41
Merisier	46,94034175	2,231967067	04/09/05	Aviso	32	1090	155	2,5	6806	41
Le Bois St M	46,91738055	2,150815473	28/08/05	Kosto	66	1164	155	4,5	5995	32
La Motte	46,9490471	2,218034081	03/09/05	Kosto	41	1125	155	2,21	5985	40
Brossats	46,97828473	2,150407253	31/08/05	Aviso	52	780	152	2,42	7225	37
Verdières	46,98306942	2,06816512	29/08/05	Aviso	36	485	173	0,6	7467	42
La Vigne	46,9498977	2,078840936	29/08/05	Kosto	15	1115	155	1,9	5947	40,65
Les Vergnes	46,96885697	2,162005014	27/08/05	Kosto	37	1285	167	1,66	6290	31
Le Bout de l'A	46,97353877	2,164486975	28/08/05	Kosto	33	450				31
Les Tuileries	46,89771044	2,142943077	31/08/05	Campala	111	655	140	3,7	5000	24,5

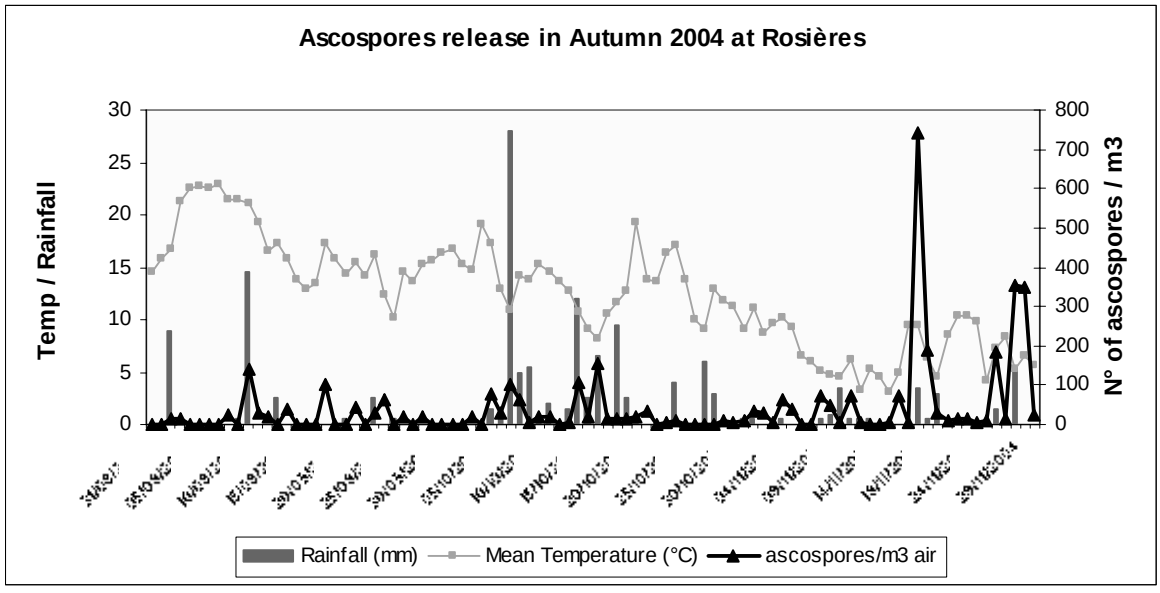


Figure 1. Ascospore release and climatic data from Rosières (East side of the working area) from beginning of September to the end of November 2004.

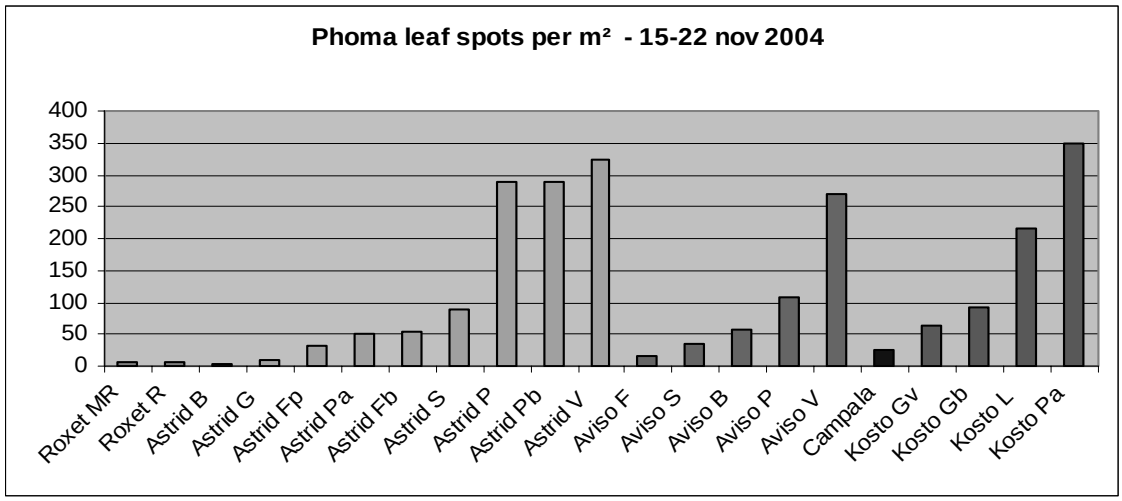


Figure 2: Phoma leaf spots at the end of November on the 21 fields observed.

As expected, the 2 fields of genotype Roxet, the Rlm7 line, have shown only a very low number of leaf spots. However, even these spots looked rather small and abnormal suggesting that in some cases the infections were probably caused by the related species *L. biglobosa*. Nevertheless, three of these isolates were virulent on 23.1.1, the reference line for Rlm7 in the differential set (Rouxel et al 2003). These isolates are currently being verified to determine any other associated virulences. A very low number of pre-existing virulent avrlm7 individuals has already been isolated from other locations in France (Balesdent et al 2006). The question is whether the virulent isolates have the ability to increase from this low number to produce a virulent population able to breakdown the major gene and how fast this process might occur under selection pressure.

The G2 disease index scores were generally low for almost all the fields with only three of fields achieving a score higher than 3 on a scale from 1 to 9. The genotypes used are known for their good tolerance to the disease. Although the major gene Rlm9 has already been overcome, cultivars have a strong quantitative resistance. Nevertheless variability was observed between fields for the same cultivar. One of the two fields sown with the genotype Roxet had a good score of 1.3 as might be expected from a cultivar with an efficient major gene. The other field with Roxet was good, but the score of 3.3 for this field was high and this field was one of the three fields with a G2 score of up to 3. Such a result could be explained in different ways:

- A relatively high percentage of WOSR volunteers amongst the sowing rows which could be an old and susceptible genotype.
- The presence of others pathogens or bio-aggressors: *Leptosphaeria biglobosa, Verticillium longisporum,* stem insects, which interact with the index G2 estimation.
- The presence of a few, but enough, virulent isolates able to produce symptoms. Results from ongoing isolate checks and virulence characterisation of local populations should provide an answer.

There is no strong argument that there is a high risk of break down during coming season. For 2004-05, only two lines, Roxet and Hearty (erucic line) were commercially available. In the cropping area studied, two of 20 fields were cropped with Roxet. During the present cropping year (2005-06), four lines and three hybrids were identified as having the major

resistance gene Rlm7 (Balesdent and Pinochet, personal communication). Consequently, during our second working year on the same area, half of the observed fields are cropped with Exagone, Exocet, and Extend, the promising hybrids from Monsanto. This allows us to focus on the behaviour of the Rlm7 hybrids located close to fields cropped the previous year with the line Rlm7 Roxet. Sampling will be increased in order to capture evidence of any increase of avrlm7 *L. maculans* isolates. Nevertheless, around 80% of the total area will be cropped with genotypes belonging to group 1 (Rlm9 is the only major gene present, for which the *L. maculans* population is already virulent). The high tolerance to *L. maculans* shown by genotypes like Aviso, ES Astrid or Grizzly are due to quantitative resistance factors. The new Rlm7 Monsanto hybrids represent only around 10% of the area. Nevertheless, the number of Lines or hybrids having the major gene Rlm7 is expected to increase quickly, as reflected by the new variety registrations done in France in 2005. So, the questions remain: Which bio-vigilance system can be used to catch the early emergence of any break down? Which tools could be useful to monitor this? How should any sampling strategy be implemented? In essence, this Study has just started, is well supported by the industry and will continue over the coming years.

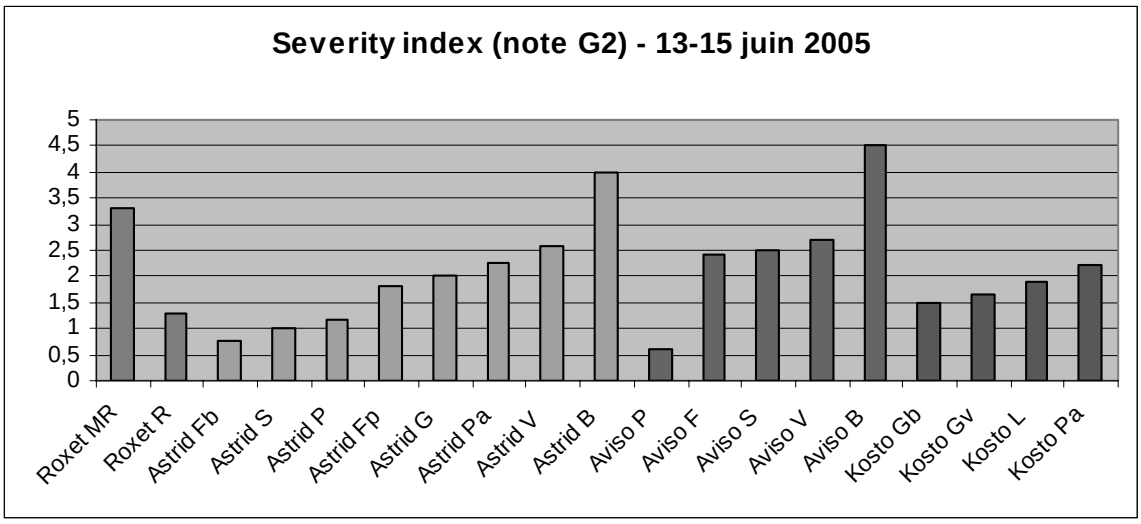


Figure 3. Final disease index G2 for the each field during the 2004/2005 growing season.

Table 2. Genotypes characterised to contain Rlm7 (Balesdent and Pinochet Pers.com.)

registration	Génotype	Breeder
2001-UK	Caïman (Line)	Monsanto
2002-UK	Roxet (Line)	Syngenta
2002-UK	Hearty (Erucic line)	Monsanto
2004-FR	Exagone (Hybrid)	Monsanto
2005-FR	Exocet (Hybrid)	DSV-Monsanto
2005-FR	Extend (Hybrid)	Monsanto
2005-FR	Quattro (Line)	Momont

Acknowledgements

This work was funded in 2004-05 by the European Union through the SECURE project (QLK5-CT-2002-01813) and in France by the plan Etat-région Centre.

References

Aubertot, J.N., Schott, J.J., Penaud, A., Brun, H. & Doré, T. 2004. Methods for sampling and assessment in relation to the spatial pattern of phoma stem canker (*Leptosphaeria maculans*) in oilseed rape. – European Journal of Plant Pathology 110:183-192.

Balesdent, M.H., Louvard, K., Pinochet, X. & Rouxel, T. 2006. A large-scale survey of races of *Leptosphaeria maculans* ocurring on oilseed rape in France. – European Journal of Plant Pathology. 114:53-65.

Brun, H., Levivier, S., Ruer, D., Somda, I., Chèvre, A.M. & Renard, M. 2000. A field method for evaluating the potential durability of new resistance sources: application to the *Leptosphaeria maculans/Brassica napus* pathosystem. – Phytopathology 90: 961-966.

CETIOM novembre 2005: Guide de l'expérimentateur colza. – Edition CETIOM, ISBN: 2-908645-64-5.

Peres, A., Poisson, B., Penaud, A., Jain, L. & Pilorge, E. 1999. *Leptosphaeria maculans* (*Phoma lingam*): A summary of three years of epidemiological studies (1995, 1996, and 1997). – Proceedings of the 10th International Rapeseed Congress, Canberra, Australia.

Pierre, .JG. & Regnault, Y. 1982. Contribution à la mise au point d'une méthode de plein champ destinée à mesurer la sensibilité des variétés de colza au Phoma. – Informations Techniques du CETIOM 81: 3-18.

Pinochet, X., Balesdent, M.H., Salvi, F., Mestries, E. & Rouxel, T. 2004. Identification of specific plant resistance factors to Phoma (*Leptosphaeria maculans*) among winter oilseed rape varieties: interest for variety testing and for the promotion of a first step of a durable management of resistances. – IOBC/wprs Bulletin 27 (6): 101-108.

Rouxel, T., Penaud, A., Pinochet, X., Brun, H., Gout, L., Delourme, R., Schmit, J. & Balesdent, M-H. 2003. A ten-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the *Rlm1* resistance gene of oilseed rape. – European Journal of Plant Pathology 109: 871-881.

West, J.S., Biddulph, J.E., Fitt, B.D.L. & Gladders, P. 1999. Epidemiology of *Leptosphaeria maculans* in relation to forecasting stem canker severity on winter oilseed rape in the UK. – Annals of Applied Biology 135: 535-546.

West, J.S., Balesdent, M.H., Rouxel, T., Narcy, J.P., Huang, Y.J., Roux, J., Steed, J.M., Fitt, B.D.L. & Schmit, J. 2002. Colonization of winter oilseed rape tissues by A/Tox+ and B/Tox° *Leptosphearia maculans* (Phoma stem canker) in France and England. – Plant Pathology 51: 311-321.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 307-316

Effects of temperature on *Rlm6*-mediated resistance to *Leptosphaeria maculans* in *Brassica napus*

Yongju Huang [1], Renhu Liu [1], Ziqin Li [1, 2], Neal Evans [1], Anne-Marie Chèvre[3], Regine Delourme [3], Michel Renard [3] and Bruce D.L. Fitt [1]

[1] *Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK*

[2] *Current address: Plant Protection Institute, Inner Mongolia Academy of Agricultural Sciences, Huhhot, 010031, China*

[3] *INRA, UMR, APBV, BP 35327, 35653 Le Rheu Cedex, France*

Abstract: Near-isogenic lines of *Brassica napus* lines with/without the resistance gene *Rlm6* were used to investigate the effects of temperature on *Rlm6*-mediated resistance to *Leptosphaeria maculans*. Leaves were inoculated with ascospores of *L. maculans* carrying the corresponding avirulence gene *AvrLm6*. Inoculated plants were incubated at 15°C or 25°C. DarmorMX (with *Rlm6*) was resistant to *L. maculans* at 15°C but susceptible at 25°C, and Darmor (without *Rlm6*) was susceptible at both temperatures. On Darmor, large grey leaf lesions developed at both 15 and 25°C. On DarmorMX, small dark necrotic spots were produced at 15°C but large grey lesions were produced at 25°C. The incubation period of *L. maculans* (from inoculation to the appearance of first lesion) was longer on DarmorMX than on Darmor. The infection efficiency (number of lesions resulting from inoculation with 100 ascospores) was greater on Darmor than on DarmorMX at 15 and 25°C. Further characterisation of the *Rlm6*-mediated resistance showed that the resistance was effective at 25°C if inoculated plants were pre-incubated at 15°C for 5 days before moving them to 25°C, but not if inoculated plants were pre-incubated at 15°C for only 2 days before moving them to 25°C. We conclude that temperature affects *Rlm6*-mediated resistance to *L. maculans* in *B. napus* leaves.

Key words: Resistance gene, avirulence gene, oilseed rape, phoma stem canker/blackleg

Introduction

Effects of temperature on host plant resistance response have been reported in several pathosystems. Tobacco *N* gene mediated resistance to tobacco mosaic virus (TMV) is temperature-sensitive. Below 27°C, the *N* resistance gene mediates a hypersensitive response (HR); above 27°C there is no HR and the virus is able to infect the plant systemically (Wright *et al*., 2000). Wheat cultivars with the resistance gene *Sr6* are resistant to the fungal pathogen *Puccinia graminis* f.sp. *tritici* (stem rust) at 18°C but susceptible at 27°C (Moerschbacher *et al*., 1989). Tomato *Cf-4* gene mediated resistance to *Cladosporium fulvum* (leaf mould) is also sensitive to temperature. Transformed tomato seedlings expressing both the *Cf-4* gene and the corresponding avirulence gene *Avr4* died rapidly as a result of systemic HR at 20°C but grew normally at 33°C (de Jong *et al*., 2002). The HR is commonly associated with a gene-for-gene interaction. *Leptosphaeria maculans*, causing phoma stem canker of oilseed rape, has a gene-for-gene interaction with *Brassica napus* (Ansan-Melayah *et al*., 1998; Balesdent *et al*., 2001). Several single locus resistance genes (e.g. *Rlm1, Rlm4, Rlm7, Rlm9*) (Ansan-Melayah *et al*., 1998; Rouxel *et al*., 2003; Delourme *et al*., 2004) and the corresponding avirulence genes (*AvrLm1, AvrLm4, AvrLm7, AvrLm9*) have been identified (Balesdent *et al*., 2002, 2005). However, little is known about the influence of temperature on interactions between *B. napus* resistance genes and *L. maculans* avirulence genes.

Although both ascospores and conidia are produced during epidemics of phoma stem canker on oilseed rape, a disease of worldwide importance (Fitt *et al*., 2006), phoma stem canker epidemics are initiated by air-borne ascospores (West *et al*., 1999; Huang *et al*., 2005). However, most previous studies on resistance to *L. maculans* have been done with conidial inoculum. When cotyledons (cv. Quinta) were inoculated with *L. maculans* conidia (race A2 isolates), the response was resistant at 18°C but susceptible at 27°C (Badawy *et al*., 1992). The interaction between Quinta and A2 isolates of *L. maculans* has now been genetically characterised as a specific *Rlm1/AvrLm1* interaction (Ansan-Melayah *et al*., 1995; Balesdent *et al*., 2001). In these experiments using conidial inoculum, leaves had to be wounded before inoculation. This methodology is not ideal for studying interactions between a pathogen and its host, since wounding alone can induce plant defence responses. The development of an ascospore inoculation method (i.e. simulating natural infection; Huang *et al*., 2006) now provides an appropriate method to investigate the effects of temperature on the interaction between *L. maculans* and *B. napus*. A major resistance gene *Rlm6*, originating from *B. juncea*, has been introgressed into *B. napus* (Chèvre *et al*., 1997; Barret *et al*., 1998) and the corresponding avirulence gene (*AvrLm6*) has been identified and partially characterised (Balesdent *et al*., 2002, 2005). However, this new resistance gene has not been used in commercial cultivars and it is not clear whether environmental factors affect the effectiveness of this resistance gene. This paper describes experiments to investigate effects of temperature on phenotypic expression of *Rlm6*-mediated resistance to *L. maculans* in *B. napus* leaves and strategies for optimising the use of this resistance gene are discussed.

Materials and methods

Four experiments were done in controlled environment cabinets, using *B. napus* near isogenic lines Darmor (lacking *Rlm6*) and DarmorMX (carrying *Rlm6*) (Huang et al., 2005) to avoid potential effects associated with host genetic background. Ascospores of *L. maculans* with the corresponding avirulence gene *AvrLm6* were used as inoculum for these experiments.

Preparation of plant material and inoculum

Plants of Darmor and DarmorMX were grown in pots (5 cm diameter) containing peat-based compost and a soluble fertiliser. Plants were initially grown in a glasshouse (20–23°C) with one plant per pot and placed in seed trays (37 cm × 23 cm) with 14 plants (seven plants of Darmor and seven plants of DarmorMX) per tray. Three weeks after sowing, the plants were transferred to a 15°C controlled environment cabinet (12 h light/ 12 h darkness, light density 210 μe $m^{-2}s^{-1}$) until each plant had three expanded leaves (GS 1,3; Sylvester-Bradley & Makepeace, 1985) and was ready for inoculation.

L. maculans ascospore inoculum was obtained from naturally infected winter oilseed rape stem base debris. Winter oilseed rape (cv. Lipton) stem bases (< 5 cm above ground level, including tap roots) with basal phoma canker were collected after harvest from fields at Rothamsted, UK in August 2000. It was confirmed that these stem pieces produced only ascospores of *L. maculans* (Huang *et al*., 2003). The stem pieces containing mature pseudothecia were stored dry at -20°C until required. To determine whether the ascospores produced on these stem base debris were appropriate to study the phenotypic expression of *Rlm6*-mediated resistance, 25 single ascospore isolations were made from the debris and tested for pathogenicity on DarmorMX and Darmor. All these isolates were avirulent on DarmorMX and virulent on Darmor; thus the ascospores from that debris carried the avirulence gene *AvrLm6*. Further characterisation of the *L. maculans* population indicated that the frequency of the *AvrLm6* allele in the natural population at Rothamsted is 100%

(Stachowiak *et al*., 2006). Therefore, the debris was used as a source of ascospore inoculum for these experiments.

Inoculation and disease assessment

In experiments 1–3, plants were inoculated with air-borne *L. maculans* ascospores (Huang *et al*., 2006). To inoculate the plants, pieces of stem base debris bearing mature pseudothecia were removed from storage at -20°C, cut into pieces 2–3 cm long and thoroughly mixed. Six stem pieces were chosen at random and evenly attached to the underside of a tray lid (37 cm × 23 cm × 14 cm) with Vaseline (Chesebrough-Pond's Ltd, London). The pieces of stem were sprayed with distilled water until run-off to induce release of ascospores and the lids with attached pseudothecia were placed over the trays with plants to let ascospores naturally deposit onto the plants at 15°C. After 2 h, the pieces of debris with pseudothecia were removed from the tray lids. The inoculated plants were sprayed with distilled water using a laboratory sprayer and immediately covered with tray lids and moved to a 15 or 25°C growth cabinet. After 48 h, the tray lids were removed. To estimate the number of ascospores deposited per unit leaf area, three glass microscope slides (7.5 cm × 2.5 cm) were placed in between the plants in each tray at approximately the same height as the leaves. The number of ascospores deposited on each slide was counted to estimate number of spores deposited per cm^2. The maximum length and width of each leaf on ten plants of each line were measured just before inoculation to estimate the leaf area per plant, then the number of ascospores deposited per plant was calculated [total leaf area (cm^2) per plant × number of spores per cm^2]. The number of lesions resulting from inoculation with 100 ascospores was then estimated [(total number of lesions per plant) ÷ (total number of spores deposited per plant) × 100].

In experiment 4, plants were inoculated with droplets of *L. maculans* ascospore suspension. Ascospore suspension was prepared using the method described by Huang *et al*. (2003) and the concentration of ascospores was adjusted to 10^3 ascospore ml^{-1} using a haemocytometer slide. Before inoculation, the leaf surfaces were slightly rubbed with a cylindrical eraser so that they would retain drops of suspension. Then a 20 µl droplet of ascospore suspension was placed on each rubbed site. Two sites (one site on each half of the leaf along the main vein) on the first and second leaf of each plant were inoculated. Five plants each of Darmor or DarmorMX were inoculated for each treatment. Plants were covered with tray covers immediately after inoculation and kept for 48 h to maintain high humidity for each treatment. There were six treatments in this experiment: (1) inoculated plants of Darmor incubated continuously at 15°C for 12 days; (2) inoculated plants of DarmorMX incubated continuously at 15°C for 12 days; (3) inoculated plants of Darmor incubated continuously at 25°C for 12 days; (4) inoculated plants of DarmorMX incubated continuously at 25°C for 12 days; (5) inoculated plants of DarmorMX incubated at 15°C for 2 days then moved to 25°C for 10 days; (6) inoculated plants of DarmorMX incubated at 15°C for 5 days then moved to 25°C for 7 days.

In experiments 1–3, the numbers of new large (> 2 mm diameter) grey phoma leaf lesions on Darmor (at 15 and 25°C) and DarmorMX (at 25°C), or small (< 2 mm diameter) dark spots on DarmorMX at 15°C, were counted daily until no new lesions (or dark spots) appeared or the leaf senesced. The time from inoculation until the first lesion/spot appeared (incubation period) was recorded. The infection efficiency was estimated as the number lesions/spots resulting from inoculation with 100 ascospores. In experiment 4, the time from inoculation until the first lesion/spot appeared was recorded. The maximum length and width of each lesion/spot were measured 12 days after inoculation.

Statistical analysis

In experiments 1–3, analyses of variance were done to assess the effects of temperature on incubation period, number of lesions and infection efficiency on DarmorMX (*Rlm6*) and Darmor (lacking *Rlm6*). In experiment 4, analyses of variance were done to assess the effects of treatments on lesion size. All the analyses were done using GENSTAT statistical software (Payne *et al*., 1993).

Results

Phoma leaf spot phenotype

Disease symptoms differed between 15 and 25°C on DarmorMX (carrying *Rlm6*), but not on Darmor (lacking *Rlm6*), whether the plants were inoculated using air-borne ascospores or droplets of ascospore suspension. Large grey lesions (> 2mm in diameter; typical grey phoma leaf lesions) developed on Darmor at both 15 and 25°C (Fig. 1a,b). On DarmorMX at 15°C, small dark necrotic spots (Fig. 1a) observed by 14 days after inoculation remained small (< 2 mm in diameter), even when leaves senesced. However, at 25°C large grey lesions developed on DarmorMX (Fig.1b).

Incubation period and number of lesions

When leaves were inoculated with air-borne ascospores, temperature ($P < 0.001$) affected the incubation period of *L. maculans*, estimated as the time from inoculation to the appearance of the first typical grey lesions on Darmor or small dark spots (15°C)/grey lesions (25°C) on DarmorMX. The incubation period was 1.3 and 7 days longer at 15°C than at 25°C for Darmor and DarmorMX, respectively (Table 1). The incubation period was also longer for DarmorMX than for Darmor ($P < 0.001$). At 15°C, 11 days after inoculation, large grey lesions had developed on Darmor. Visible symptoms were not observed on DarmorMX until 13-14 days after inoculation. The incubation period of *L. maculans* on Darmor and DarmorMX was shorter when leaves were inoculated with droplets of ascospore suspension than when leaves were inoculated with air-borne ascospores. For example, the times from inoculation to the first lesion on Darmor at 15 and 25°C were 6 and 4 days for inoculation with ascospore droplets, compared to 8 and 6 days for inoculation with air-borne ascospores.

Temperature ($P < 0.01$) affected the number of grey phoma lesions (Darmor or DarmorMX at 25°C)/small dark necrotic spots (DarmorMX at 15°C). At both 15 and 25°C, more lesions developed on Darmor than did spots/lesions on DarmorMX ($P < 0.001$). On Darmor, more lesions developed at 15°C (52.9) than at 25°C (35.7) (Table 1). On DarmorMX, the number of small dark spots which developed at 15°C was less than the number of large grey lesions which developed at 25°C. Temperature affected the infection efficiency, estimated as number of lesions/spots produced by inoculation with 100 ascospores on leaves of Darmor and DarmorMX (Table 1). The infection efficiency was greater on Darmor than DarmorMX ($P < 0.001$) at both 15°C and 25°C. On leaves of Darmor and DarmorMX, all the sites inoculated with a drop of ascospore suspension produced lesions (on Darmor at 15 and 25°C and on DarmorMX at 25°C) or spots (on DarmorMX at 15°C).

Table 1. Effects of temperature on incubation period, number of lesions and infection efficiency on leaves of *Brassica napus* near isogenic lines (NIL) Darmor (lacking *Rlm6*) or DarmorMX (carrying *Rlm6*) inoculated with ascospores of *Leptosphaeria maculans* carrying the avirulence gene *AvrLm6*

Infection parameter[a]	NIL	15°C	25°C	SED[d] (4 df)
Incubation period[b]	Darmor	7.3	6.0	0.3
	DarmorMX	13.7	6.7	
Number of lesions/spots per plant	Darmor	52.9	35.7	7.2
	DarmorMX	3.1	12.4	
Infection efficiency[c]	Darmor	40.2	12.2	9.3
	DarmorMX	2.3	4.4	

[a] Data presented are means from experiments 1–3.
[b] The incubation period was estimated as the time (day) from inoculation to appearance of first large grey lesions on Darmor or small dark spots (15°C)/grey lesions (25°C) on DarmorMX.
[c] Infection efficiency was estimated as the number of lesions/spots produced by inoculation with 100 ascospores [(no. lesions) ÷ (no. ascospores) × 100].
[d] Approximate maximum SED.

Effects of temperature shift on lesion development

In experiment 4, large grey typical phoma leaf lesions (> 2mm in diameter) developed on Darmor at both 15 and 25°C at 12 days after inoculation. Small dark necrotic spots (< 2 mm in diameter) were observed on DarmorMX at 15°C and large grey lesions at 25°C, but the lesion area on DarmorMX was smaller than that on Darmor (Fig. 2). However, after pre-incubation of DarmorMX at 15°C for 5 days before incubation at 25°C for 7 days, the small dark necrotic spots which developed and did not change into large grey lesions. The area of necrotic spots on DarmorMX pre-incubated for 5 days at 15°C before incubation at 25°C was smaller than the area of grey lesions on DarmorMX incubated continuously at 25°C for 12 days (Fig. 2). However, after pre-incubation of DarmorMX at 15°C for 2 days before incubation at 25°C for 10 days, no small dark necrotic spots were observed but large grey lesions developed. The lesion area on DarmorMX (either pre-incubated or not pre-incubated) was smaller than the lesion area on Darmor (Fig. 2).

Discussion

Results of these experiments suggest that temperature affects the phenotypic expression of *Rlm6*-mediated resistance to *L. maculans* in *B. napus* leaves. On Darmor (lacking *Rlm6*), there were no differences in the type of symptom between 15 and 25°C; typical large grey lesions developed at both 15 and 25°C, regardless of the inoculation method. However, there were differences between temperatures in the type of symptom on DarmorMX (carrying *Rlm6*). Small dark necrotic spots developed on DarmorMX at 15°C, whereas large grey lesions developed at 25°C whether the plants were inoculated with air-borne ascospores or droplets of ascospore suspension. The differences in symptom development on DarmorMX

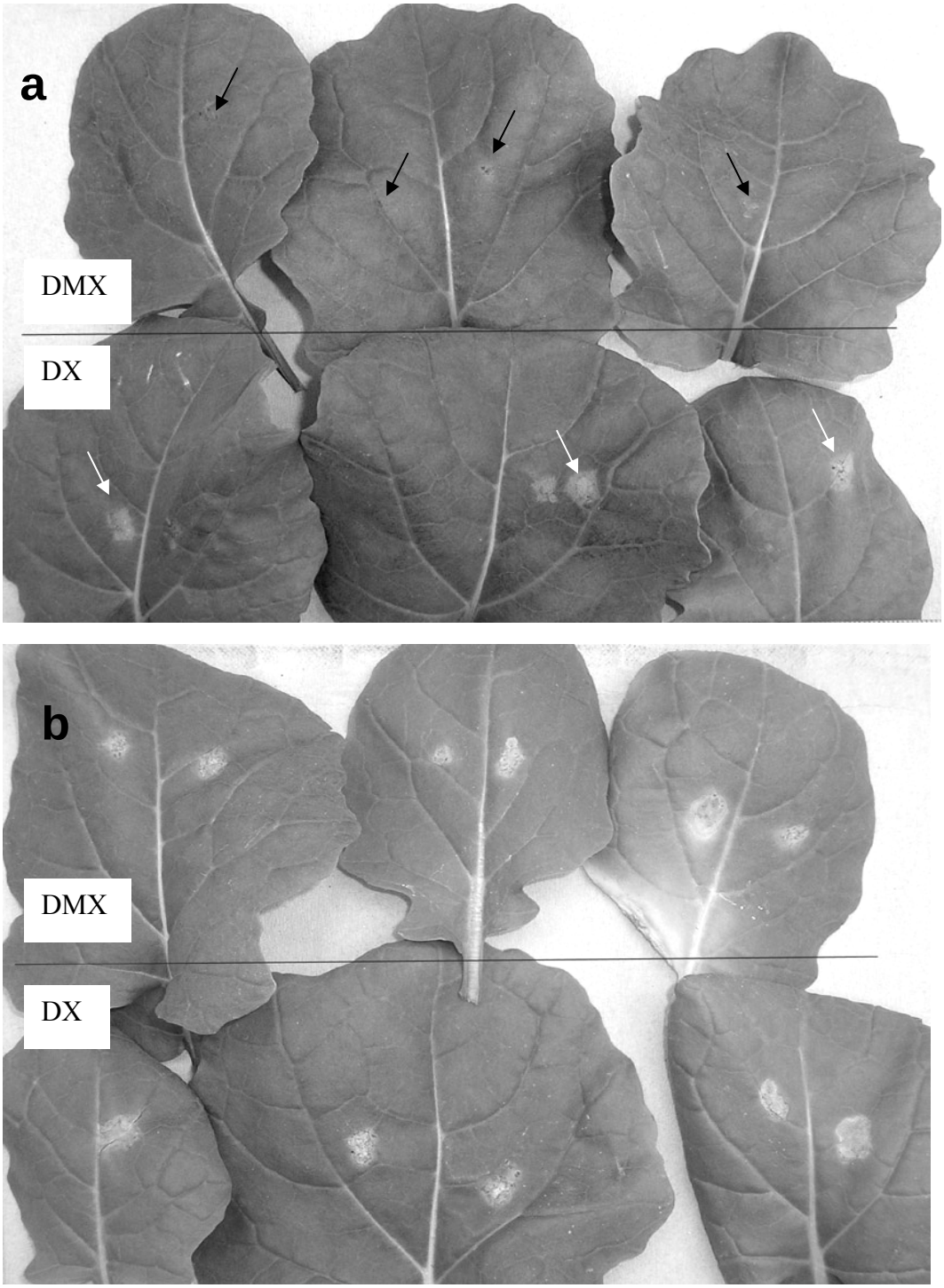


Figure 1. Symptoms on leaves of *Brassica napus* near isogenic lines Darmor (lacking *Rlm6*) and DarmorMX (carrying *Rlm6*) inoculated with droplets of ascospore suspension of *Leptosphaeria maculans* carrying the corresponding avirulence gene *AvrLm6*, 12 days after inoculation in experiment 4. (a) large grey lesions (white arrows) on Darmor (DM) and small dark necrotic spots (black arrows) on DarmorMX (DMX) at 15°C; (b) large grey lesions on Darmor and DarmorMX at 25°C.

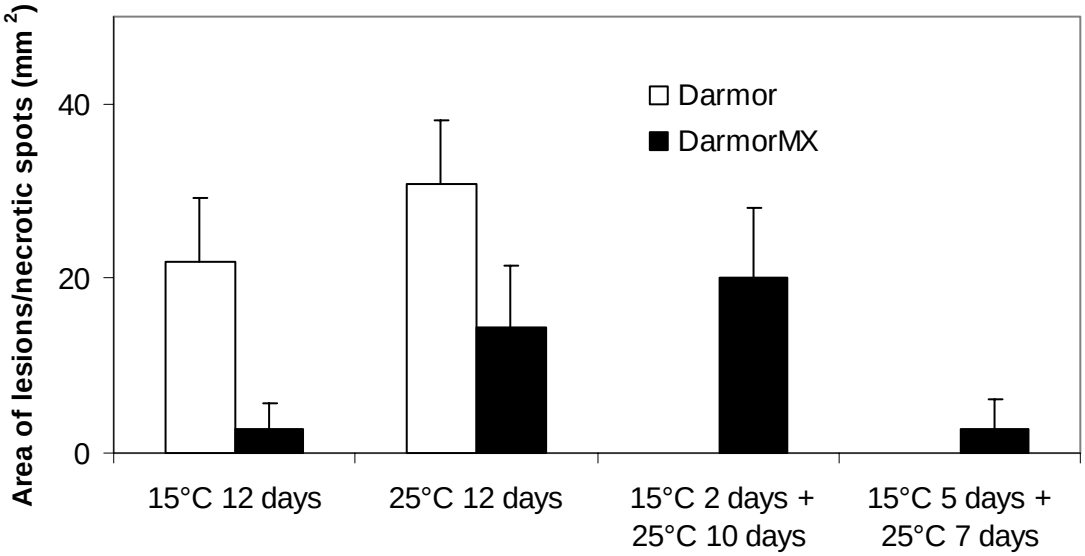


Figure 2. Differences in areas of phoma leaf spot lesions/spots on leaves of *Brassica napus* near isogenic lines Darmor (lacking *Rlm6*, □) and DarmorMX (carrying *Rlm6*, ■) inoculated with droplets of ascospore suspension of *Leptosphaeria maculans* carrying the corresponding avirulence gene *AvrLm6*, between treatments in experiment 4 involving continuous incubation at 15°C or 25°C, or a combination of these temperatures. Vertical lines are SED (19 df). Two types of symptom: phoma leaf lesions (#) and small dark spots (*) were observed.

between temperatures suggest that temperature may affect interactions between *L. maculans* avirulence genes and *B. napus* resistance genes. Although temperature-sensitivity of major gene resistance to viruses (Wright *et al*., 2000), bacteria (Ullrich et al., 2000) and biotrophic fungal pathogens (Moerschbacher *et al*., 1989) has been reported, this is the first report of temperature-sensitivity of major gene resistance to a hemi-biotrophic fungal pathogen, such as *L. maculans*.

These results also suggest that temperature affects the probability that an ascospore deposited on a leaf surface will produce a small dark necrotic spot (15°C) or large grey lesion (25°C) on DarmorMX or large grey lesion on Darmor (15 and 25°C). For *L. maculans* to colonise the host tissue successfully, ascospores first need to germinate and hyphae/germ tubes from ascospores need to penetrate leaf surfaces. The fact that the number of lesions which developed on Darmor was greater than on DarmorMX at 15 and 25°C suggests that the probability that an ascospore depositing on a leaf surface can germinate, penetrate and successfully colonise the leaf tissue is greater on Darmor than on DarmorMX. That small dark necrotic spots developed on DarmorMX at 15°C but grey lesions developed at 25°C suggests that ascospores depositing on leaf surfaces can germinate and hyphae from ascospores can penetrate the leaf tissue of DarmorMX. However, they suggest that *L. maculans* fails to colonise the tissue at 15°C whereas it can successfully colonise the tissue at 25°C. This suggests that the crucial stage when temperature affects *Rlm6*-mediated resistance to *L. maculans* occurs after penetration. This hypothesis needs to be tested in further experiments.

Results obtained suggest that pre-incubation of inoculated DarmorMX at 15°C for 5 days before transfer to 25°C can prevent colonization by *L. maculans*, since a necrotic reaction was observed and these necrotic spots did not develop into large grey lesions during subsequent incubation at 25°C. However, pre-incubation of DarmorMX at 15°C for 2 days before transfer

to 25°C did not prevent the colonization by *L. maculans* since large grey lesions developed during subsequent incubation at 25°C. The difference in the resistance response of inoculated plants at 25°C between those with a 2-day and those with a 5-day pre-incubation at 15°C suggests that resistance to *L. maculans* in DarmorMX may develop between 2 and 5 days after inoculation. This topic needs future investigation.

The influence of temperature on the effectiveness of *Rlm6* against *L. maculans* may explain why phoma stem canker of oilseed rape is most severe in Australia, where temperatures during the growing season are higher than in Europe (minimum/maximum mean daily temperature during oilseed rape growing seasons are 6°C/30°C and 1°C/21°C in West Australia and West Europe, respectively) (West *et al*., 2001). Results of these controlled environment experiments indicate that *Rlm6*-mediated resistance to *L. maculans* is not effective at 25°C; similarly *Rlm1*-mediated resistance to *L. maculans* is not effective at 27°C (Badawy *et al*., 1992). If oilseed rape cultivars with such temperature-sensitive resistance genes are deployed in areas where the temperatures are lower, such as the UK, there will be less risk of severe stem canker epidemics than in areas where the temperatures are greater, such as Australia. Furthermore, the work suggests that, should there be rapid climate change with increasing temperature in Europe (Thuiller *et al*., 2005), there is a potential for more severe phoma stem canker epidemics. Therefore, there is a need to investigate the mechanisms by which temperature affects the interactions between *L. maculans* avirulence genes and *B. napus* resistance genes, to develop integrated strategies for management of these limited plant resistance sources and decrease the risk that novel resistance sources will be rapidly overcome.

Acknowledgements

We thank the European Union (QLRT-2001-01813, SECURE project), the UK Department for Environment, Food and Rural Affairs (Defra, OREGIN project, AR0703), the Biotechnology and Biological Sciences Research Council, the Chadacre Agricultural Trust and the Royal Society for supporting the work. We thank Akinwunmi Latunde-Dada for helpful discussion and Alan Todd for statistical analyses of the data.

References

Ansan-Melayah, D., Balesdent, M.H., Buèe, M. & Rouxel, T. 1995: Genetic characterization of *AvrLm1*, the first avirulence gene of *Leptosphaeria maculans*. – Phytopathology 85: 1525-1529.

Ansan-Melayah, D., Balesdent, M.H., Delourme, R., Pilet, M.L., Tanguy, X., Renard, M. & Rouxel, T. 1998: Genes for race-specific resistance against blackleg disease in *Brassica napus* L. – Plant Breeding 117: 373-378.

Badawy, H.M.A., Kakau, J. & Hoppe, H.H. 1992: Temperature and ageing of host tissue affect the interactions between different oilseed rape cultivars and pathotype groups of *Leptosphaeria maculans*. – Journal of Phytopathology 134: 255-263.

Balesdent, M.H., Attard, A., Ansan-Melayah, D., Delourme, R., Renard, M. & Rouxel, T. 2001: Genetic control and host range of avirulence towards *Brassica napus* cvs Quinta and Jet Neuf in *Leptosphaeria maculans*. – Phytopathology 91:70-76.

Balesdent, M.H., Attard, A., Kühn, M.L. & Rouxel, T. 2002: New avirulence genes in the phytopathogenic fungus *Leptosphaeria maculans*. – Phytopathology 92: 1122-1133.

Balesdent, M.H., Barbetti, M.J., Li, H., Sivasithamparam, K., Gout, L. & Rouxel, T. 2005: Analysis of *Leptosphaeria maculans* race structure in a worldwide collection of isolates. – Phytopathology 95: 1061-11071.

Barret, P., Guérif, J., Reynoird, J., Delourme, R., Eber, F., Renard, M. & Chèvre, A. 1998: Selection of stable *Brassica napus-Brassica juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*). 2. A 'to and fro' strategy to localise and characterise interspecific introgressions on the *B. napus* genome. – Theoretical and Applied Genetics 96: 1097-1103.

Chèvre, A.M., Barret, P., Eber, F., Dupuy, P., Brun, H., Tanguy, X. & Renard, M. 1997: Selection of stable *Brassica napus-B. juncea* recombinant lines resistant to blackleg (*Leptosphaeria maculans*) 1: Identification of molecular markers, chromosomal and genomic origin of the introgression. – Theoretical and Applied Genetics 95: 1104-1111.

de Jong, C.F., Takken, F.L.W., Cai, X., de Wit, P.J.G.M. & Joosten, M.H.A.J. 2002: Attenuation of *Cf*-mediated defence responses at elevated temperatures correlates with a decrease in elicitor-binding sites. – Molecular Plant-Microbe Interactions 15: 1040-1049.

Delourme, R., Pilet-Nayel, M.L., Archipiano, M., Horvais, R., Tanguy, X., Rouxel, T., Brun, H., Renard, M. & Balesdent, M,H. 2004: A cluster of major specific resistance genes to *Leptosphaeria maculans* in *Brassica napus*. – Phytopathology 94: 578-583.

Fitt, B.D.L., Brun, H., Barbetti, M.J. & Rimmer, S.R. 2006: World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). – European Journal of Plant Pathology 114: 3-15.

Huang, Y.J., Toscano-Underwood, C., Fitt, B.D.L., Hu, X.J. & Hall, A.M. 2003: Effects of temperature on ascospore germination and penetration of oilseed rape (*Brassica napus*) leaves by A-group or B-group *Leptosphaeria maculans* (phoma stem canker). – Plant Pathology 52: 245-255.

Huang, Y.J., Fitt, B.D.L., Jedryczka, M., Dakowska, S., West, J.S., Gladders, P., Steed, J.M. & Li, Z.Q. 2005: Patterns of ascospore release in relation to phoma stem canker epidemiology in England (*Leptosphaeria maculans*) and Poland (*L. biglobosa*). – European Journal of Plant Pathology 111: 263-277.

Huang, Y.J., Evans, N., Li, Z.Q., Eckert, M., Chèvre, A.M., Renard, M.& Fitt, B.D.L. 2005: Temperature and leaf wetness duration affect phenotypic expression of *Rlm6*- mediated resistance to *Leptosphaeria maculans* in *Brassica napus*. – New Phytologist: (in press).

Huang, Y.J., Li, Z.Q., Evans, N., Rouxel, T., Fitt, B.D.L. & Balesdent, M.H. 2006: Fitness cost associated with loss of the *AvrLm4* avirulence function in *Leptosphaeria maculans* (phoma stem canker of oilseed rape). – European Journal of Plant Pathology 114: 77-89.

Moerschbacher, B.M., Witte, U., Königs, D. & Reisener, H.J. 1989: Changes in the level of enzyme activities involved in lignin biosynthesis during the temperature sensitive resistant response of wheat (Sr6) to stem rust (P6). – Plant Science 65: 183-190.

Payne, R.W., Lane, P.W., Digby, P.G.N., Harding, S.A., Leech, P.K., Morgan, G.W., Todd, A.D., Thompson, R., Tunnicliffe, W.G., Welham, S.J. & White, R.P. 1993. Genstat 5 Release 3 Reference Manual. – Oxford, UK: Clarendon Press.

Rouxel, T., Penaud, A., Pinochet, X., Brun, H., Gout, L., Delourme, R., Schmit J. & Balesdent, M.H. 2003: A 10-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the Rlm1 resistance gene of oilseed rape. – European Journal of Plant Pathology 109: 871-881.

Stachowiak, A., Olechnowicz, J., Jedryczka, M., Rouxel, T., Balesdent, M.H., Happstadius, I., Gladders, P., Latunde-Dada, A.O. & Evans, N. 2006: Frequency of avirulence alleles in field populations of *Leptosphaeria maculans* in Europe. – European Journal of Plant Pathology 114: 67-75.

Sylvester-Bradley, R. & Makepeac,e R.J. 1985: Revision of a code for stages of development in oilseed rape (*Brassica napus* L.). – Aspects of Applied Biology (Field Trials Methods and Data Handling) 10: 395-400.

Thuiller, W., Lovorel, S., Araújo, M.B., Sykes, M.T. & Prentice, I.C. 2005: Climate change threats to plant diversity in Europe. – Proceedings of the National Academy of Sciences of USA 102: 8245-8250.

Ullrich, M.S., Schergaut, M., Boch, J. & Ullrich, B. 2000: Temperature-responsive genetic loci in the plant pathogen *Pseudomonas syringae* pv. *glycinea*. – Microbiology 146: 2457-2468.

West, J.S., Biddulph, J.E., Fitt, B.D.L. & Gladders, P. 1999: Epidemiology of *Leptosphaeria maculans* in relation to forecasting stem canker severity on winter oilseed rape in the UK. – Annals of Applied Biology 135: 535-546.

West, J.S., Kharbanda, P.D., Barbetti, M.J. & Fitt, B.D.L. 2001: Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. – Plant Pathology 50: 10-27.

Wright, K.M., Duncan, G.H., Pradel, K.S., Carr, F., Wood, S., Oparka, K.J. & Cruz, S.S. 2000: Analysis of the N gene hypersensitive response induced by a fluorescently tagged tobacco mosaic virus. – Plant Physiology 123: 1375-1385.

In vitro comparison of fitness of *AvrLm1* vs. *avrLm1* isolates of *Leptosphaeria maculans*

Julia Olechnowicz [1], Marie-Hélène Balesdent [2] and Malgorzata Jędryczka [1]
[1] *Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland*
[2] *National Institute of Agronomic Research, Versailles, France*

Abstract: One of the main diseases of rapeseed (*Brassica napus*) in Europe and across the world is stem canker of crucifers, caused by *Leptosphaeria maculans (Phoma lingam).* Stem canker contributes to great losses of winter oilseed rape yield. The newly introduced resistance of cultivars can be overcome by the pathogen within a few years.

Experiments in controlled temperature environment were performed to compare growth rate of near isogenic isolates of *L. maculans*, differing with avirulence gene towards resistance gene *Rlm 1*. The studies were done using six avirulent isolates (*AvrLm1*) and six virulent isolates (*avrLm1*). The isolates were cultivated on six media: Malt Extract Agar (MEA), Luria Broth Agar (LBA), Water Agar (WA), Potato Dextrose Agar (PDA), Campbell's V8 Juice Agar (V8) and Synthetic low Nutrient Agar (SNA). Twenty ml of medium was poured on a 9 mm diameter Petri dish. Experiments were performed in controlled environment at 20 ^{o}C and 12 hour photoperiod. Three replicates for each variant (isolate x medium) were used. Growth rate was measured every two days from 3 days to 3 weeks after depositing the fungus on the medium.

Statistically significant differences between growth rate of virulent and avirulent isolates were observed at the early phase of the experiment – three days after subculturing fungi on media V8, PDA and LBA and 5 days after subculturing on V8, with avirulent isolates growing faster than virulent ones. The fastest growth rate was observed on V8 medium and the slowest growth was on MEA medium. One week after depositing an agar disc on a medium, differences between avirulent and virulent isolates were no longer significant, but avirulent isolates tended to grow faster on most of the media.

The experiment confirms the hypothesis of loss of fitness connected with the gain of new virulence of an isolate. Similar results were previously reported for *AvrLm4* vs. *avrLm4* isolates of *L. maculans*, although the differences between two groups of isolates were mostly observed *in vivo*.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 319-322

Detection and quantification of *Leptosphaeria maculans* in the leaf petiole of *Brassica napus*

Elizabeth Pirie[1], Akinwunmi O. Latunde-Dada[1], Peter Werner[2] and Bruce D.L. Fitt[1]
[1]Rothamsted Research, Harpenden, Herts, U.K. AL5 2JQ; [2]CPB-Twyford, 56 Church Street, Thriplow, Nr Royston, Herts, U.K. SG8 7RE

Abstract: In controlled environment experiments winter oilseed rape (*Brassica napus*) cultivars Canberra and Courage did not differ significantly in leaf lesion size and systemic growth in petioles, measured after inoculation of leaves with conidia of *Leptosphaeria maculans* isolate ME24 (transformed to express green fluorescent protein). In field experiments (2003/2004) quantification of *L. maculans* within petioles of six doubled haploid (DH) lines of oilseed rape derived from Canberra x Courage showed differences in the number of colony forming units obtained from maceration of petiole parts. Differences between lines did not relate to differences in stem canker severity at harvest.

Key words: *GFP*, phoma stem canker, resistant cultivars, systemic growth

Introduction

The pathogen *Leptosphaeria maculans* produces damaging epidemics of phoma stem canker in the oilseed rape crop world-wide (Fitt *et al.*, 2006). Initial leaf lesion formation from infection by air-borne ascospores is followed by a period of asymptomatic, systemic growth down the petiole to the stem where the pathogen can cause a damaging canker (West *et al.*, 2001). In the UK, stem canker severity is assessed in trials to provide a measure of field resistance of cultivars, which is published in the HGCA Recommended List (http://www.hgca.com). This assessment, made before harvest, encompasses all types of resistance, tolerance and disease avoidance which have occurred during the 11 month growing season. To understand the components of field resistance, more detailed analysis must be done. Qualitative, gene-for-gene resistance operates in *B. napus* at the phoma leaf spotting stage, but this resistance is easily overcome when cultivars with only this form of resistance are grown intensively. Quantitative resistance may be more durable but has proven more difficult to study. To determine where components of resistance to *L. maculans* may act within oilseed rape cultivars several techniques have been employed. Using the *gfp* (green fluorescent protein) gene from the jellyfish *Aequorea victoria* to transform the pathogen to constitutively express GFP, visualisation of the pathogen infection pathway and life cycle can be achieved (Eckert *et al.*, 2005). This paper describes work done to compare two cultivars (Canberra and Courage) that differ in field resistance (resistant and susceptible) using GFP transformed *L. maculans* to compare lesion size and systemic growth. Doubled haploid lines from a Canberra x Courage mapping population were compared by quantifying *L. maculans* in the petiole.

Materials and methods

Controlled environment experiments

Lesion size
Leaf 2 of five plants each of winter oilseed rape cvs Canberra and Courage was detached and placed on 9 cm diameter plates of sterile distilled water agar (DWA). Each leaf was wounded with a sterile needle on either side of the mid-rib, and inoculated with a 10µl drop containing 10^6 conidia/ml of *L. maculans* isolate ME24. Plates were sealed with Parafilm M (American National Can Company, USA) and placed in a controlled environment chamber (18^{o}C day/15^{o}C night, 12 h photoperiod). The diameter of 10 lesions per cultivar was measured after 14 days.

Systemic growth
Leaf 2 of 10 plants each of cvs Canberra and Courage was inoculated with a 10µl drop containing 10^6 conidia/ml of *L. maculans* isolate ME24 expressing GFP, after wounding with a sterile needle. After inoculation, plants were maintained within magenta vessels (Sigma) in a controlled environment chamber (18^{o}C day/15^{o}C night, 12 h photoperiod). A Leica MZ FLIII stereo-microscope (Leica Microsystems, Milton Keynes, UK) equipped with filters GFP1 (excitation 425/60nm, emission 480nm) and GFP3 (470/40nm, 525/50nm) was used to observe the distance *L. maculans* had grown down the petiole, and this was measured, starting from the base of the leaf lamina, 20 days after inoculation.

Field experiment

Maceration and plating
Samples for this experiment were taken from 100 doubled haploid (DH) lines, originating from Canberra x Courage (CPB-Twyford), grown in the field at Pinchbeck, Spalding in the 2003-2004 growing season. Samples of 3 petioles per line (leaf 8) were taken on 4 March 2004 and stored at -20^{o}C. Six lines were selected on the basis of stem canker scores on 7 July 2004 for quantification of *L. maculans* in the petiole. Three lines had high (3-4) stem canker scores and three lines had low (0-1) stem canker scores (scored on a 0-4 scale; 0, no disease; 1, less than half the stem girdled; 2,more than half the stem girdled; 3,whole stem girdled; 4, plant dead (Zhou *et al.*, 1999)). Petioles were divided into two parts, the half nearest the leaf (upper) and the half nearest the stem (lower). Petiole parts were ground separately in 10 ml sterile distilled water with sterile sand and 4 drops of 20% lactic acid added to prevent bacterial contamination. The resulting macerate was diluted 100 times with sterile distilled water and 1 ml of macerate was spread on a 9 cm plate of DWA, with 3 replicates. The resulting number of colonies was counted after 10 days.

Results and discussion

Lesion size
There was no significant difference in lesion size observed between cvs Canberra and Courage (Figure 1). This suggests that the field resistance to *L. maculans* of cv. Canberra, as suggested by its HGCA Recommended List rating, does not operate at the leaf lesion stage of the *L. maculans* life cycle.

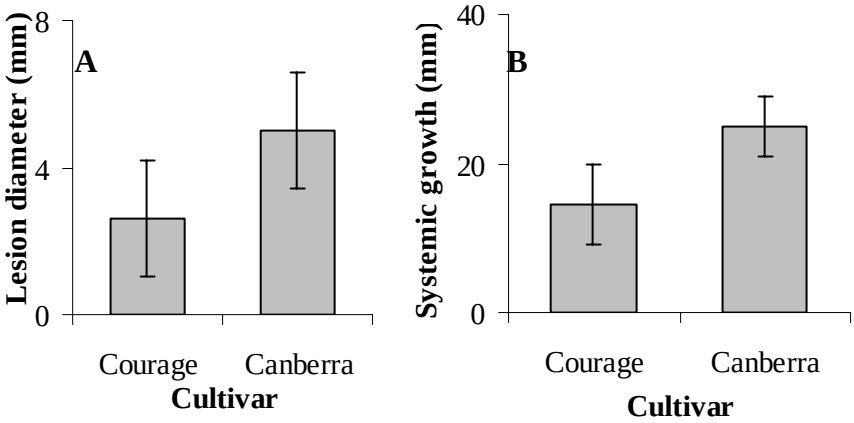


Figure 1. **A** Phoma leaf lesion size on two cultivars of winter oilseed rape 14 days after inoculation of leaf 2 with *L. maculans* (GFP expressing isolate ME24) conidia in a controlled environment (SED = 1.59, df = 3). **B** Systemic growth (mm) measured down the petiole from the leaf lamina of leaf 2 on two cultivars 22 days after inoculation of leaves with *L. maculans* (GFP expressing isolate ME24) conidia in a controlled environment (SED = 7.51, df = 3).

Systemic growth

No significant difference in systemic growth of *L. maculans* between cvs Canberra and Courage was observed 20 days after inoculation (Figure 1). This suggests the absence of differential host responses to the systemic growth of the *L. maculans* isolate in petioles of leaf 2 from both cultivars. Further studies of the growth of the pathogen in the petiole may show differences at later stages of systemic infection.

In these controlled environment experiments, use of a larger number of plants might have detected significant differences between the two cultivars. These experiments will therefore be scaled up. Furthermore, interpretation of results must take into account that controlled environments do not always reproduce field conditions.

Maceration and plating

There were differences in the number of fungal colonies obtained from the six lines and between upper and lower petiole parts (Figure 2), but the sample size was low. The upper petiole parts had more colony forming units than lower parts. This may be due to differences in the extent of pathogen growth from the leaf. Differences between lines in the number of colony forming units in the petiole of these leaves did not relate to differences in stem canker severity measured before harvest, in summer. An earlier leaf sample, in autumn, may have shown different results. This technique has no specificity for *L. maculans* so the colonies counted may have been other fungi. A more specific method for detection of *L. maculans*, such as quantitative PCR, is needed to determine the quantity of pathogen in the petiole. Studies on the usefulness of quantitative PCR for assessing resistance to *L. maculans* in oilseed rape cultivars have already been initiated and the quantity of pathogen determined in autumn in parts of the petiole seems to correlate well with severity of stem canker in the following summer (Kenyon *et al.*, 2004).

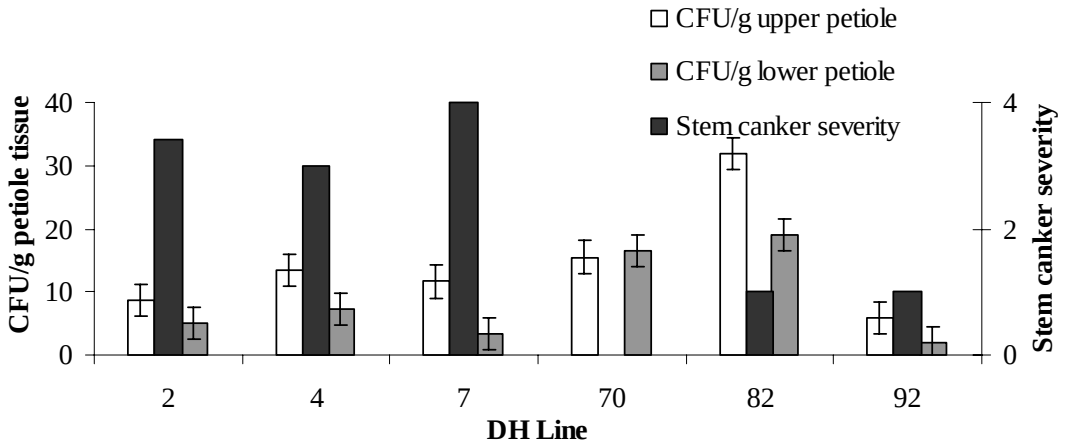


Figure 2. Differences in amount of fungal colonies (CFU/g) in upper and lower parts of petioles of leaf 8 between six naturally infected DH winter oilseed rape lines sampled on 4 March 2004 at Pinchbeck (P<0.05, df = 48, SED = 3.64, n=3) in relation to differences in stem canker severity on 7 July 2004, measured on a 0-4 scale (0, no disease; 1, less than half the stem girdled; 2, more than half the stem girdled; 3,whole stem girdled; 4, plant dead). Data are means of 5 plants per plot.

Acknowledgements

The authors would like to thank the Home Grown Cereals Authority and the Biotechnology and Biological Sciences Research Council for funding, CPB Twyford for the Canberra x Courage DH lines and Dr. P Spanu for advice. All work with transformed isolates of *L. maculans* was done under Defra plant health license PHL174C/4952 (11/2004).

References

Eckert, M., Maguire, K., Urban, M., Foster, S., Fitt, B., Lucas, J. & Hammond-Kosack, K. 2005: *Agrobacterium tumefaciens*-mediated transformation of *Leptosphaeria* spp. and *Oculimacula* spp. with the reef coral gene DsRed and the jellyfish gene gfp. FEMS – Microbiology Letters 253: 67-74.

Fitt, B.D.L., Brun, H. Barbetti, M.J. & Rimmer, R.S. 2006: World-wide importance of phoma stem canker (*Leptosphaeria maculans* and *L. biglobosa*) on oilseed rape (*Brassica napus*). – European Journal of Plant Pathology 114:3-15.

Kenyon, D., Thomas, J. & Handy, C. 2004: Feasibility of using quantitative PCR for assessing resistance to stem canker in oilseed rape cultivars. – IOBC/wprs Bulletin 27 (6): 109-117.

West, J.S., Kharbanda, P.D. Barbetti, M.J. & Fitt, B.D.L. 2001: Epidemiology and management of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. – Plant Pathology 50: 10-27.

Zhou, Y., Fitt, B.D.L., Welham, S.J., Gladders, P., Sansford, C.E. & West, J.S. 1999: Effects of severity and timing of stem canker (*Leptosphaeria maculans*) symptoms on yield of winter oilseed rape (*Brassica napus*) in the UK. – European Journal of Plant Pathology 105: 715-728.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 323-328

Impact of rape stem weevil, *Ceutorhynchus napi*, on the early stem infection of oilseed rape by *Phoma lingam*

Ulrike Krause, Birger Koopmann and Bernd Ulber
Department of Crop Science, Plant Pathology and Plant Protection, University of Göttingen, Grisebachstrasse 6, D-37077 Göttingen, Germany

Abstract: The rape stem weevil, *Ceutorhynchus napi* Gyll., is one of the most destructive insect pests of winter oilseed rape (*Brassica napus*) in Central Europe. Females deposit their eggs into the top of elongating stems mainly in March and April, thereby inducing substantial changes of stem growth like swelling, deformation and splitting. Moreover, wounding of plant tissue by egg-laying females and stem-mining larvae is thought to predispose the stems to early secondary infections by fungal pathogens, particularly *Phoma lingam.*

To test the hypothesis that *C. napi* is capable of increasing early stem infections by *P. lingam* at the time of oviposition, the relation between the number of oviposition punctures on stems and the incidence of *P. lingam* within the stem pith was determined. In addition, controlledexperiments were dome under standardised conditions in the laboratory to determine whether the fungal inoculum is being transmitted by *C. napi* females. Results showed that the severity of *P. lingam* stem infection increased significantly with increasing number of oviposition punctures of *C. napi*. Experiments in the laboratory provided evidence for the transmission of *P. lingam* by *C. napi*.

Key words: *Ceutorhynchus napi*, *Phoma lingam*, oviposition punctures, stem infection, disease transmission

Introduction

During recent years the damage potential of pests and diseases in winter oilseed rape crops has increased. Severe economic losses can be caused by insect pests and fungal pathogens. Plant damage by the rape stem weevil, *Ceutorhynchus napi* (*Col., Curculionidae*) has been reported throughout all Central-European rape-growing areas, with yield losses up to 50% (Alford et al., 2003). To deposit their eggs into plants from March onwards, females of *C. napi* bite deep punctures into the growing tissue close to the tip of elongating stems. This causes growth distortions like bending, swelling and splitting of stems (Le Pape & Bronner, 1987; Lerin, 1993). The larvae cause extensive feeding channels within the stem pith. Five to six weeks later, the full-grown larvae leave the stem through exit holes to pupate in the soil. The stem tissue damaged by stem-mining larvae has been observed frequently to be associated with secondary fungal pathogens, particularly *Phoma lingam* (Broschewitz et al., 1993). In the past, higher severity of *Phoma* stem infection has been attributed to larval feeding galleries and exit holes in stems (Newman, 1984). Stems infested by *C. napi* larvae showed higher severities of upper stem lesions than those without weevil infestation (Broschewitz et al., 1993). However, no information was available on the impact of ovipositing punctures of *C. napi* on early stem infection by *P. lingam*.

This study was conducted to determine the role of *C. napi* on stem infections by *P. lingam*, from the beginning of egg-laying in spring. We have investigated whether feeding wounds by egg-laying females provide points of entry for the pathogen and whether the fungal inoculum is transmitted by females from plant to plant.

Methods and results

Field experiments

To examine the relationship between plants showing oviposition punctures and early stem infection by *P. lingam*, samples of 50 plants were collected from each of three crops of winter oilseed rape in the course of the oviposition period of *C. napi* on 7 April, 19 April and 5 May 2005. Sample sites were located north of Göttingen, Germany. The sampled crops (Weendelsgraben [WG], cv. 'Express'; Weendelsbreite [WB], cv. 'Express'; Tannenberg [TB], cv. 'Talent') were cultivated according to common agricultural practise, but no insecticides were applied in spring. On each plot, five infested plants were selected at random from 10 points. In the laboratory, plant sections of 0.5 to 2 cm containing either oviposition punctures or splits arising from oviposition punctures were cut from all stems. The stem sections were incubated in moist chambers. For the identification of *P. lingam*, the outgrowing mycelium was transferred to V8 agar plus 200 ppm Streptomycin for pycnidia and pycnidiospore production.

To relate the number of oviposition punctures by *C. napi* to disease severity of *P. lingam*, the symptoms of *P. lingam* on stems and root collars were examined by using the rating scale established by Krüger (1979). In each plot, a total of 100 plants was scored on 15 May, 9 June and 1 July. At the same time, the number of oviposition spots on stems was counted.

Laboratory experiments

To investigate the potential for *C. napi* to transmit *P. lingam* propagules, controlled experiments were done under standardised conditions in the laboratory. Cotyledons of potted oilseed rape plants (cv. 'Lirabon', DSV, Lippstadt, Germany) were inoculated with an A-Type isolate of *P. lingam* T12aD34 (Kuswinanti et al., 1999). On each cotyledon 20 µl of a 10^7 ml^{-1} conidia suspension was applied. From 10 to 14 dpi, when *Phoma* lesions had produced numerous pycnidia, mated females of *C. napi* were caged individually right on the lesion by using small clip cages (Ø 1.5 cm). Other females were caged on non-inoculated cotyledons with no access to *Phoma* inoculum. Females had been collected from winter oilseed rape crops (WB, WG, TB) in March and stored at 6°C. Following two days of feeding, each female was transfered to an elongating stem of potted oilseed rape plants (cv. 'Mozart', NPZ, Hohenlieth, Germany; BBCH 33) for oviposition. The plants were reared in a growth chamber under a photoperiod of 16h light and 8h dark at 19°C. After two more weeks, the number of oviposition punctures on the shoots was counted. Subsequently, small sections of stem tissue (0.5 to 2 cm) containing oviposition punctures were cut out and incubated in a moist chamber. To investigate whether *P. lingam* infection had occurred within the pith, any outgrowing mycelium was transferred to V8 agar for pycnidia and pycnidiospore production.

Results and discussion

Field experiments

Among the plants infested by *C. napi* which had been sampled at three occasions from three crops of oilseed rape, the number of plants infected by *P. lingam* increased over time (Tab.1). On 7 April, *P. lingam* was detectable only from 0.7 % of all plants. On 19 April and 5 May, the mean percentage of infected plants increased to 38% and 47%, respectively. At WB and WG, the percentage of infected stems was higher than at TB. On 5 May the proportion of *P. lingam* isolates in stem sections containing oviposition punctures ranged between 84.6 % and 89.7 %. In contrast, stem sections containing splits accounted only for 10.3 % to 15.4 % of *P. lingam* infections.

Table 1: Disease incidence of *P. lingam* on oilseed rape plants injured by oviposition punctures and splits of *C. napi.* Samples of 50 plants were collected from each of three crops of winter oilseed rape (WB, WG, TB).

	Sampling site and date								
	7 April			19 April			5 May		
	WB	WG	TB	WB	WG	TB	WB	WG	TB
Number of infected plants	0.0	1.0	0.0	23.0	27.0	9.0	28.0	29.0	13.0
infections around punctures (%)	0.0	100.0	0.0	82.6	70.4	55.6	85.7	89.7	84.6
infections around splits (%)	0.0	0.0	0.0	17.4	29.6	44.4	14.3	10.3	15.4

The results show that the early stem infection by *P. lingam* was highly related to the presence of oviposition punctures by *C. napi*. The small number of infected plants at sample site TB may have resulted from the smaller numbers of oviposition punctures per plant. At TB the seed had been treated with the insecticide 'Chinook' (imidacloprid + beta-cyflutrin) and the fungicide 'Folicur' (tebuconazole ;0,7 l/ha) had been applied at growth stage BBCH 39. These chemical treatments resulted in healthier, vigorously growing plants as well as in higher plant density which is known to reduce the number of oviposition punctures by *C. napi* (Nuss, 2004). The low incidence of *Phoma* infections around splits may have resulted from a fast wound-reaction and desiccation combined with a small fungal inoculum in the air. Furthermore, splits (located mainly at upper stem positions) may get infected by the non-aggressive, B-Typ of *P. lingam (L. biglobosa,* Shoemaker and Brun, 2001*)*, which causes only superficial necrotic lesions (Thürwächter et al., 1999; West et al., 2002).

Table 2: Relationship between the level of oviposition punctures by *C. napi* and the severity of *P. lingam* on stems and root collars of oilseed rape (means of WB, WG and TB). *Phoma* scored according to Krüger (1979): 1= no infection; 3= larger spots on the surface with light lignification; 5= deeper infection at the stem; 7= profound infection with pycnidia; 9= plant dead, little connection to the root

Date	% plants attacked by *C. napi*	Mean no. of oviposition punctures per plant (± SD)	Mean *P. lingam* score root collar (± SD)	Mean *P. lingam* score stem (± SD)	Correlation (r) *P. lingam* stem – punctures[1]
15 May	92.0	9.3 ± 6.4	1.8 ± 1.1	2.2 ± 1.1	0.72**
9 June	98.0	11.1 ± 6.3	2.8 ± 1.6	4.0 ± 2.2	0.59**
1 July	96.0	-	2.9 ± 1.5	4.9 ± 2.1	-

[1] (**p < 0,01; df =299)

Among the plants which were scored for oviposition punctures and symptoms of *Phoma* stem infection following the oviposition period in mid May to early July, 92 %– 98 % showed

oviposition punctures, the mean number of punctures per plant increased from 9.3 % to 11.1% on 15 May and 9 June, respectively (Tab. 2). Disease severity of *P. lingam* was scored according to Krüger (1979) at both, the root collar and the upper stem. The severity of *Phoma* on stems was higher than on root collars, both of them increasing over time. As *C. napi* does not oviposit into the root collar, it has no impact on the infection of the root collar. The results emphasises the impact of oviposition by *C. napi* for P*homa* stem lesions. There is a significant regression between the score for *P. lingam* stem infection and the number of oviposition punctures (Fig. 1).

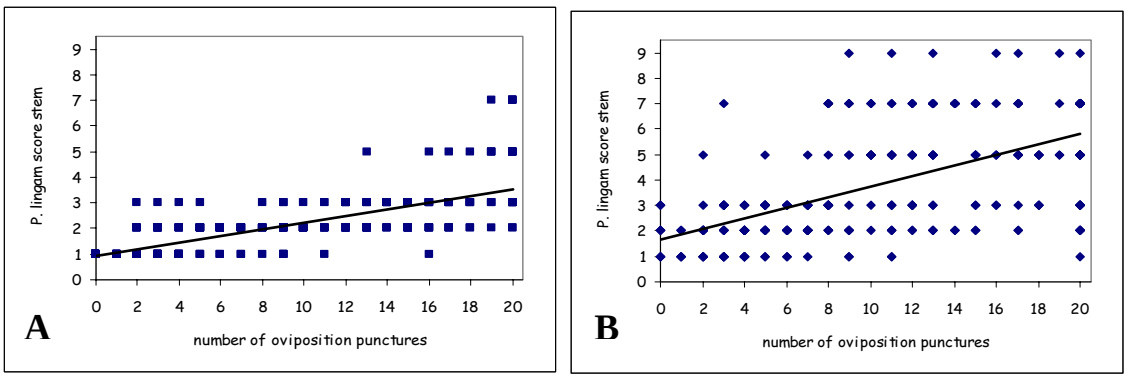


Fig. 1: Regression between the number of oviposition punctures and the severity of *P. lingam* (Krüger, 1979; see Table 2) on 300 stems of oilseed rape. A: 15 May. y = 0.13x + 0.92, R^2 = 0.52, **p < 0.01, df = 299; B: 9 June. y = 0.21x + 1.67, R^2 = 0.35, **p < 0.01; df = 299.

Table 3: Incidence of *P. lingam* transmission to stems of oilseed rape (n = 30) by ovipositing females of *C. napi* under laboratory conditions

No. oviposition punctures/plant	*C. napi* previously exposed to **infected** cotyledons		*C. napi* previously exposed to **non-infected** cotyledons	
	no. of plants	% *P. lingam* infected stems	no. of plants	% *P. lingam* infected stems
0	2.0	0.0	1.0	0.0
< 10	6.0	33.3	9.0	0.0
≥ 10	22.0	50.0	20.0	0.0

Laboratory experiments

The observations in the field were confirmed under standardised conditions in the laboratory, where females of *C. napi* were shown to transmit the spores of *P. lingam* from infected cotyledons to stems of oilseed rape plants. With increasing number of oviposition punctures, the incidence of plants infected by *P. lingam* increased (Tab. 3). Plants showing no oviposition punctures were not infected. Plants with less than ten oviposition punctures showed a *Phoma* incidence of 33.3%. On plants with ten and more punctures, *P. lingam* infection was more likely, with a frequency of 50%. Female *C. napi* previously exposed to non-infected cotyledons did not cause any infection by *P. lingam*. In conclusion, when female

C. napi previously exposed to sporulating lesions of *P. lingam* were used as the only source inoculation of stems, the frequency of transmission of the pathogen was very high.

The lab experiments provided further evidence that the incidence of stem infection by *P. lingam* increases significantly with oviposition of *C. napi*. Our results correspond to findings by Gaudet & Schulz (1981) who found that another weevil, *Apion occidentale*, is able to transmit the causal agent of *Phoma* black stem disease (*Phoma oleracea* var. *helianthi-tuberosi*) of sunflowers. We assume that *P. lingam* propagules adhering to the rostum of *C. napi* females are responsible for the early transmission of *P. lingam* to the oilseed rape stems during the oviposition period. To confirm the potential of *C. napi* for transmission of *P. lingam* and to prove the contamination of outer integuments and gut channels of field collected weevils by *Phoma* propagules, further tests are being performed. Although late stem infections by *P. lingam* via the exit holes of *C. napi* larvae during flowering might occur (Broschewitz et al., 1993), these presumably are of minor importance for the damage of *P. lingam* to oilseed rape. It is evident from our experiments that the early transmission of fungal propagules by ovipositing females of *C. napi* has a stronger impact on *Phoma* incidence than the secondary infection of stems following damage by larvae.

References

Alford, D.V., Nilsson, C. & Ulber, B. 2003: Insect pests of oilseed rape crops. – In: Alford, D.V. (ed.). Biocontrol of oilseed rape pests. Blackwell Publishing, Oxford, UK: 9-41.

Broschewitz, B. Steinbach, P. & Goltermann, S. 1993: Einfluß stengelbewohnender tierischer Schaderreger auf den Befall von Winterraps mit *Phoma lingam* und *Botrytis cinerea*. – Gesunde Pflanzen 45: 106-110.

Gaudet, M.D. & Schulz, J.T. 1981: Transmission of *Phoma oleracea* var. *helianthi-tuberosi* by the adult stage of *Apion occidentale*. – Journal of Economic Entomology 74: 486-489.

Krüger, W. 1979. Verbreitung der Wurzelhals- und Stengelfäule (verursacht durch *Phoma lingam*) bei Raps in der Bundesrepublik Deutschland. – Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 31: 145-148.

Kuswinanti, T. Koopmann, B. & Hoppe, H.-H. 1999: Virulence pattern of aggressive isolates of *Leptosphaeria maculans* on an extended set of *Brassica* differentials. – Journal of Plant Diseases and Protection 106: 12-20.

Lerin, J. 1993: Influence of the growth rate on the splitting of the stem after an attack of *Ceutorhynchus napi* Gyll.. – IOBC/wprs Bulletin 16 (: 160-163.

Le Pape, H. & Bronner, R. 1987: The effect of *Ceuthorrhynchus napi* (Curculionidae, Coleoptera) on stem tissues of *Brassica napus* var *oleifera*. In: Proceedings of the 6th International Symposium on Insect-Plant Relationships, eds. Labeyrie, Fabres, Lachaise: 207-212.

Newman, P.L. 1984: The effects of insect larval damage upon the incidence of canker in winter oilseed rape. Proceedings of the British Crop Protection Conference – Pests and Diseases – 1984: 815-822

Nuss, H. 2004: Einfluss der Pflanzendichte und –architektur auf Abundanz und innerpflanzliche Verteilung stängelminierender Schadinsekten in Winterraps. Ph.D. Thesis, University of Göttingen.

Shoemaker, R. A. and H. Brun 2001: The teleomorph of the weakly aggressive segregate of *Leptosphaeria maculans*. Canadian Journal of Botany 79(4): 412-419.

Thürwächter, F. Garbe, V. Hoppe, H.-H. 1999: Ascospore Discharge, Leaf Infestation and Variations in Pathogenicity as Criteria to Predict Impact of *Leptosphaeria maculans* on Oilseed Rape. Journal of Phytopathology 147: 215-222.

West, J.S. Kharbanda, P.D. Barbetti, M.J. Fitt, B.D.L. 2001: Epidemiology and management of of *Leptosphaeria maculans* (phoma stem canker) on oilseed rape in Australia, Canada and Europe. Plant Pathology 50: 10-27.

West, J.S. Balesdent, M.-H. Rouxel, T. Narcy, J.P. Huang, Y.-J. Roux, J. Steed, J.M. Fitt, B.D.L. Schmit, J. 2002: Colonization of winter oilseed rape tissue by A/Tox+ and B/Tox0 *Leptosphaeria maculans* (phoma stem canker) in France and England. Plant Pathology 51: 311-321.

The complete-genome sequencing project of *Leptosphaeria maculans*

Marie-Hélène Balesdent [1], Barbara J. Howlett [2] and Thierry Rouxel [1]
[1] Institut National de la Recherche Agronomique, PMDV, Route de St-Cyr, 78026 Versailles Cedex, France, [2] School of Botany, the University of Melbourne, VIC 3010 Australia

Abstract: Until now *Leptosphaeria maculans*, which is responsible for major losses on oilseed rape crops, has been poorly characterized at the genomic level. This fungus belongs to the dothideomycete class, which encompasses numerous important phytopathogenic fungi, such as the wheat pathogen *Stagonospora nodorum*, the apple scab agent *Venturia inaequalis*, and the oilseed rape pathogen *Alternaria brassicicola*. The species belonging to this group share some common life traits, such as their mode of infection and colonization, the frequent production of secondary toxic metabolites, or the frequent occurrence of a sexual stage in their life cycle. They also differ by their host range, and include pathogens of both monocots and dicots. The whole genome sequence of *S. nodorum* was very recently released, while genome initiatives for the whole sequencing of *L. maculans* at Genoscope, and *A. brassicicola* in the USA were recently successful. Therefore the complete sequences of three closely related phytopathogenic fungi will soon be available. In this communication, the *L. maculans* genome initiative will be presented, along with the perspectives in terms of new fields of research offered by these sequence data. In particular, comparative genomic studies between *L. maculans*, *S. nodorum* and *A. brassicicola* are expected to allow identification of pathogenicity genes either common or specific to each of these plant/fungus interactions. Also, the complete sequence of *L. maculans* will allow the development of a large set of new micro- and minisatellite markers. These will be used both to develop population genetic studies and therefore help answer unresolved epidemiological questions, and to speed up map-based cloning of avirulence genes, and therefore help develop molecular markers of specific races of the pathogen, which will be useful for a better management of specific resistance genes. The complete sequence data will also speed up the molecular analyses of pathogenicity genes identified either via random insertional mutagenesis, or via a systematic genome-wide search of candidate genes, including avirulence genes, and genes involved in toxin production.

Significance of *Leptosphaeria maculans* and *Sclerotinia sclerotiorum* incidence of winter rapeseed in the Czech Republic

Lenka Odstrcilova [1] and Eva Plachka [2]
[1] Agritec, Research, Breeding and Services Ltd., odstrcilova@agritec.cz, [2]OSEVA PRO Ltd., Research Institute of Oilseed Crops at Opava, plachka.vuol@quick.cz

Abstract: Phoma root rot (causal agent *Leptosphaeria maculans*) and white mold (causal agent *Sclerotinia sclerotiorum*) are serious pathogens of oilseed rape in the Czech Republic. In test years we confirmed, the strong dependence of disease occurrence on the position of rape in crop rotation system, weather conditions, characteristics of locality and growing technology (fungicidal treatment).
In 2000 and sporadically also in 2001, the occurrence of visual symptoms of white mold attack was observed before fungicidal treatment in early spring and at the beginning of fast prolongation growth especially on plants having the overgrown symptoms at autumn

In growing season 2001/2002 heavy infestation of oilseed rape by phoma root rot was observed. In field trials, the mean disease infestation was 57 % on treated plots in comparison with 67 % on untreated controls. This result corresponds with the low effect of fungicidal treatments in autumn and early spring on increase of seed yield (+3.6 %). Infestation of oilseed rape by white mold in 2002 was about 30 %. Targeted fungicidal treatment against this disease at the full flowering stage increased seed yield by 7.65 %.

In growing season 2002/2003, heavy damage of roots occurred as a result of temperature variation in early spring. The surface of cracked roots had deep furrows. This caused the difficult evaluation of phoma root rot infestation. It was not possible by visual evaluation to differentiate if the furrows on roots were caused by the weather conditions or by fungal disease. Roots of plants from plots with lower crop density and plots untreated in autumn were most damaged. Occurrence of phoma root rot on rapeseed root crown in autumn 2002 was not observed. Infestation of phoma root rot before harvest on untreated controls was in Opava 85 %, in Šumperk 73 %. Average infestation on plots treated in autumn was in Opava 78 %, in Šumperk 50%. Infestation by white mold was not observed in Opava, in Šumperk was 7 % only at untreated crops.

Sporadic phoma infection was observed at test localities Opava and Šumperk at autumn 2003; the infection was observed on the leaves only. In 2004 (BBCH 85 - the seeds becoming brown) significant infection, appearing as dark brown or black spots at the stem base was observed. The infection of roots was minimal. The infection of controls was about 50% at Opava and 15% at Šumperk. The mean infection of crops treated by combined autumn and spring treatments: in Opava about 42% and at Šumperk 4 - 8 %. The infection of Sclerotinia on untreted control crops was Opava 21%, Šumperk 39%, the mean infection of treated variants was: Opava 5 %, Šumperk 18.5 %. First symptoms of infection were observed in the second half of June.

In autumn 2004, phoma root rot symptoms were observed only on leaves, with sporadic and medium occurrence. The dependence of disease on position of oilseed rape in the crop rotation and on locality was monitored. A similar situation was also observed in spring 2005. Phoma root rot incidence on stem bases and roots of plants has not been realized so far. Infestation by white mold in this year was very high. The early start of plant infestation was recorded, occurrence of necroses, mycelia and sclerotia was observed (24th May 2005 - Slapy u Tabora). Types of infestation were different: infestation of roots and stem base was similar to 2000 and 2001. High occurrence of stem lesions to the middle or in the second third of the plant was also observed. Mostly the main stem is infected. Infestation of lateral branches and siliques is sporadic. Evaluation of infestation by both pathogens in 2005 has not been completed and the exact data are not yet available.

This work was financially supported by the Ministry of Agriculture of the Czech Republic (Projects No. QD 1350, No. QC 0308, No. QE 1262, No. IG 46061).

Sclerotinia stem rot

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 335-341

Development of a new disease and yield loss related forecasting model for Sclerotinia stem rot in winter oilseed rape in Germany

Simone Koch[1], Sarah Dunker[1], Benno Kleinhenz[2], Manfred Röhrig[3], Hans Friesland[4] and Andreas von Tiedemann[1]

[1] *Department of Crop Sciences; Division of Plant Pathology and Crop Protection, Georg-August University Göttingen, Grisebachstraße 6, 37077 Göttingen, Germany*
[2] *Centre for Decision Support Systems in Crop Protection (ZEPP), Rüdesheimer Straße 3-4, 55545 Bad Kreuznach, Germany*
[3] *ISIP, Rhüdesheimer Straße 3-4, 55545 Bad Kreuznach, Germany*
[4] *German Meteorological Service (DWD), Bundesallee 50, 38116 Braunschweig, Germany*

Abstract: *Sclerotinia sclerotiorum* the causal pathogen of Sclerotinia stem rot is a common problem in winter oilseed rape production in Germany. Fungicide treatments against the disease are routinely carried out during flowering. Prediction systems can help to reduce the costs of oilseed rape production by predicting the necessity and exact timing of a fungicide application. The major aim of prediction systems is to reduce fungicide use and to avoid crop loss. A new prediction model for Sclerotinia stem rot has been developed, involving weather data, field specific factors and damage thresholds. Data from different field trials of the state extension services from 1994-2004 in Germany were analysed to investigate the impact of different field factors as cultivar, soil type, sowing time, crop rotation, fertilization and tillage. In laboratory experiments, the effect of the microclimate in plant cover was determined. The examination of the field specific factors showed that the disease incidence is dependent on various factors which are crop rotation, sowing time, cultivar and soil type. The temperature range for an infection with ascospores is between 7 - 22°C. The new simulation model SkleroPro was validated by a retrospective calculation using data of different field trials of the state extension services with meteorological data of the last ten years with regard to the economic efficiency of fungicide applications against *S. sclerotiorum*. SkleroPro is the first disease forecasting model for a Sclerotinia disease providing a crop-loss related, field-site and time-point specific decision support. SkleroPro will be made available to growers via the internet portal 'ISIP' in 2006.

Key words: Oilseed rape, *Sclerotinia sclerotiorum*, forecasting, validation, economic efficiency

Introduction

Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary has become a major threat for oilseed rape in Germany, its growing importance being a result of the increased intensity in cultivation of this oil crop in the last two decades. Yield losses of up to 50% due to this disease have been reported in various regions and years (Pope, 1989). In Germany, stem rot primarily occurs in areas with a short crop rotation cycle. At present, control of Sclerotinia stem rot in winter oilseed rape predominantly relies on one fungicide application at the time of flowering addressing the infection by ascospores. As resistant winter oilseed rape varieties are not available at present, prophylactic fungicide sprays at full bloom have become a widely applied practice of control, although an earlier study has shown only 33% of fungicide applications to be cost-effective (Wahmhoff, 2000). In order to reduce uneconomic fungicide treatments, a suitable crop loss and disease prediction model is necessary. The development of a disease prediction model requires comprehensive knowledge of the life cycle and the environmental conditions that favour the pathogen (Clarkson *et a*l., 2004). The

aim of this work was to develop a quantitative and yield loss related forecasting model, enabling a field-site specific and economically-based recommendation for the use of fungicides against Sclerotinia stem rot. The new model was partly derived from an earlier scientific disease simulation model, SKLERO, of the German meteorological service (Friesland, 1998 & 2000).

Materials and methods

Laboratory tests

Laboratory experiments were carried out to determine the optimal infection conditions for Sclerotinia. Stem segments were excised from 6 plants (cv. 'Heros'; growth stage 65) at 30 cm plant height including an axil with a side shoot. The six stems in each box were held in place by a grid in the box. A petal was placed on each of the axils of three stems and a 10 µl drop of ascospore suspension (5×10^4 mL spores) was placed on the petal. On the remaining three stems the ascospore suspension was placed directly on the stem axil without a petal. The stems were incubated at temperatures from 6 to 28°C in two-degree steps, at 99% RH in a controlled environment chamber in the dark. Disease severity on stems was assessed daily for 14 days.

Field experiments

In order to develop a new disease and yield loss related forecasting model, data from field trials of the German state extension services (Mecklenburg-Western Pomeria, Schleswig-Holstein, Lower Saxony, Brandenburg, Saxony, Thuringia, Rhinland-Palatinate, Bavaria) collected from 1994 to 2004 were analysed to investigate the impact of different crop production factors such as cultivar, soil type, sowing time, crop rotation, fertilisation and tillage. Analysis of variance was used to determine the impact of the field-specific factors on disease incidence. Factors having a significant impact on disease severity were candidate determinants for the field-site specific disease level. In total, more than 897 field trials were analysed for effects of year, sowing time, cultivar, crop density, N-fertilisation, soil cultivation and soil type. In addition to using historic field data, field experiments were conducted at 65 locations in nine different federal states during three seasons (2002-2004).

Field specific data such as disease incidence, date of first appearance of apothecia, date of mid-bud stage (GS 55) and yield data were collected. In 2005, SkleroPro was evaluated concomitantly in 32 field experiments. Each experiment comprised a fungicide untreated control, one treatment following the model and a farm variant with routine fungicide application at full bloom (GS 65). Plots were arranged in a randomised block design with four replicates in each experiment.

Economic validation

The simulation model SkleroPro was validated by retrospective calculations using historical data from 75 sites of field trials run by the federal states plant protection services and the associated weather data from the period 1994 to 2004. Model evaluation was done with regard to the need to spray and the economic efficiency of fungicide applications conducted during flowering and to the optimal timing of treatment. Application costs included fungicide prices plus VAT, variable application costs and losses due to tractor damage to the crop.

The calculation of the variable application costs was based on data published by the 'Association for Technology and Structures in Agriculture' (KTBL) and consisted of machine costs (standard tractor with four-wheel drive, 75-92 kW, 1000 L mounted plant spraying equipment, 15 m application width) and labour costs (labour time requirement based on 200 L per hectare application volume, field size 2 ha, average foreman wage costs). Yield losses due

to tractor damage at the time of full bloom were estimated to 2.6% per hectare. Oilseed rape produce prices were averaged for the respective growing season.

Statistical analysis

Linear regression analysis was used to quantify the relationship between the infetion hours and disease incidence. The simulation software ModelMaker was used for the development, modification and evaluation of the prediction model (Walker, 1997).

Results and discussion

Effect of temperature on ascospore infection with S. sclerotiorum

Sclerotinia disease developed most rapidly on stems at 16-22°C where the germination of spores occurred 4 days after inoculation and the disease first appeared 7-9 days later. Twelve days after inoculation the mycelium covered the whole stem. At 8-10°C and 22-24°C, disease developed more slowly and after 10 days only the germination of spores was observed. Below 7°C and above 26°C no infection and no germination of spores was observed (Fig. 1). Furthermore, it was observed that the petals play an essential role in the infection with ascospores. Twenty-three percent of ascospores germinated on the stem axis without a petal whereas 100 % of the ascospores with petals germinated on the stem axis.

The environmental conditions for the infection of the pathogen have been discribed in several earlier reports (Abawi & Grogan, 1979; Weiss *et al*., 1980). In a publication by Young (1994) the temperature range for the infection with *S. sclerotiorum* was 16-27°C. Krüger (1975) observed infections starting at 8°C at relative air humidity from 84 % to 100 %.

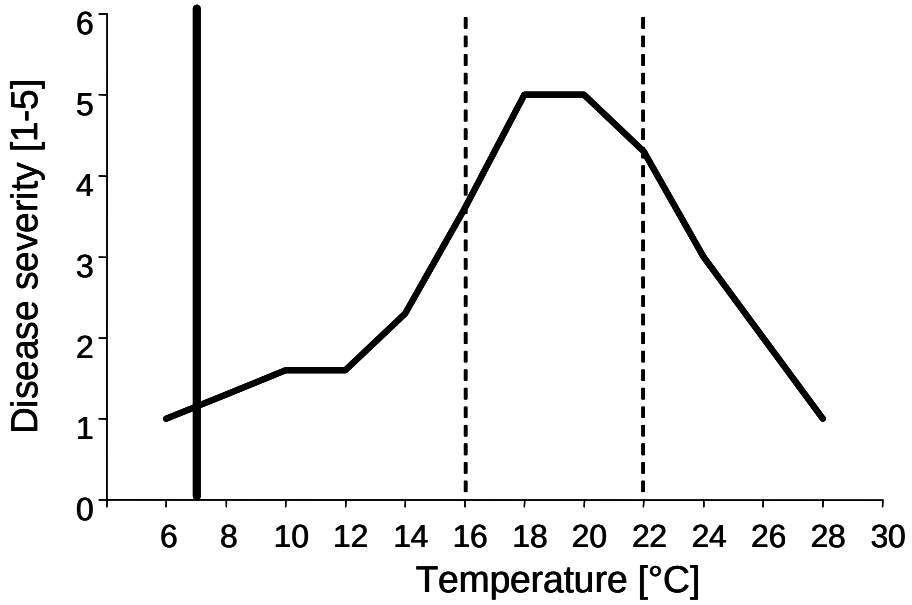


Figure 1. Ascospore infection of leaf axils with petals as related to temperature (6-28°C) at 99 % relative humidity. The temperature range for ascospore infection (6-22°C) and the minimum temperature of ascospore germination are marked (solid vertical line). Disease severity was scored as 1 = no mycelial growth, 2 = lesion restricted to infection site, 3 = lesion girdling the stem, 4 = whole stem affected, and 5 = whole stem affected with profuse mycelium.

Analysis of field specific data

The analysis of variance of historic data sets from the last ten years also revealed a significant impact of various field-site specific factors on the disease level. As a result, cultivar, sowing date, crop rotation and soil type showed a significant relationship to the incidence of *S. sclerotiorum* whereas canopy density and soil cultivation were unrelated to the disease (Tab. 1). In contrast to this Mueller *et al*. (2002) reported higher disease incidence after conventional tillage than in fields with no ploughing. At present only crop rotation is integrated as a factor in the forecasting model SkleroPro. This factor is weighted in three categories, a two year, three year and more than three year crop rotation.

Table 1. Impact of different field specific factors on disease incidence of *S. sclerotiorum.*

Field factor	n	*F*-value
N-fertilization	93	0.8 n.s.
Crop density	93	2.8 n.s.
Soil cultivation	99	0.3 n.s.
Soil type	301	8.7 **
[1]Cultivar	625	1.9 *
Crop rotation	99	4.9*
[1]Sowing time	625	3.1*
Year	549	70.4 ***

(Historic field data, n = locations in 1994–2004)
Tukey test * p<0.05; ** p<=0.01; *** p<=0.001; n.s. = not significant; [1] Wilcoxon test

SkleroPro a new forecasting model

On the basis of the results of the cropping factor analysis and the laboratory tests a new forecasting model for *S. sclerotiorum*, SkleroPro, was developed involving weather data, field-site specific factors and economic damage thresholds. Four weather variables, air temperature, humidity, rainfall and sunshine duration, were used to calculate the microclimate in the plant cover, the plant growth stage (GS) and the infection hours before and during the time of flowering. Prediction of plant developmental stages was based on temperature sums with a precision of two days between mid bud stage (GS 55) and late bloom (GS 70).

Based on controlled environment chamber data, 7°C and 80% relative humidity lasting for 23 hours were identified as minimum conditions for stem infection with ascospores. The number of infection hours exceeding 23 significantly correlated with the disease incidence at harvest time (Fig. 2).

By entering field specific parameters and the crop rotation into the model, the number of infection hours may increase, decrease or remain constant, reflecting an increase or decrease in the risk level.

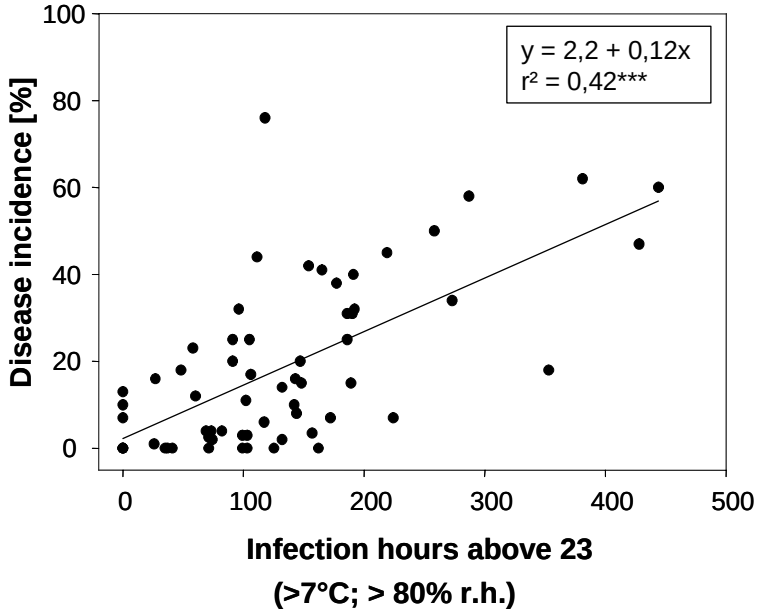


Figure 2. Function of disease incidence in relation to number of infection hours above 23 used in SkleroPro (>7°C, >80% rh), derived from data from 75 field trials done by the federal state extension services between 2000 - 2004.

SkleroPro provides two levels of prediction. The first is based on the calculated microclimate and developmental stage and gives a regional risk assessment of infection. The second determines the field specific need of fungicide use under economic terms.

When the number of 23 infection hours is exceeded, a field-specific treatment decision based on cost-effectiveness is calculated on the basis of four additional field-site specific determinants such as cost of fungicide, passage losses, rapeseed production price and yield expectation (Dunker, unpublished). These parameters and the disease infection function determine the actual threshold value of infection hours above which a treatment decision and the optimal day of treatment is indicated by the model.

SkleroPro will be made available to growers in the season 2006 via the internet portal 'ISIP' (Information Systems for Integrated Plant Production) under www.isip.de (Fig. 3).

Validation of SkleroPro

The economic analysis of the treatment decisions provided by SkleroPro based on 108 field trials done between 1994-2004 revealed a cost-effectiveness in 70% of the cases. However, in 24 % of the cases, the model overestimated disease with a final disease level being below the economic damage threshold. Underestimation occurred in 6% of the locations although the final disease exceeded the damage threshold (Fig. 4). Overall, only 35% of the routine treatments applied at these locations proved to be economic. According to Wahmhoff (2000) the average number of treatments that proved to be economic against *S. sclerotiorum* in 1981 to 1990 was 30%.

Using predictions from SkleroPro, 46 % of fungicide treatments could be saved compared to a routine application scheme. A further analysis of the model performance revealed an average yield gain of 0.5 t per hectare if the treatments followed SkleroPro predictions, resulting in an additional revenue of 32 € per hectare. The cost-effective yield gain threshold for a profitable fungicide treatment is 0.46 t per hectare. In fields sprayed using the model recommendation, the average yield gain was only 0.17 t per hectare and there were associated losses of 50 € per hectare due to the lack of cost-effectiveness of the application.

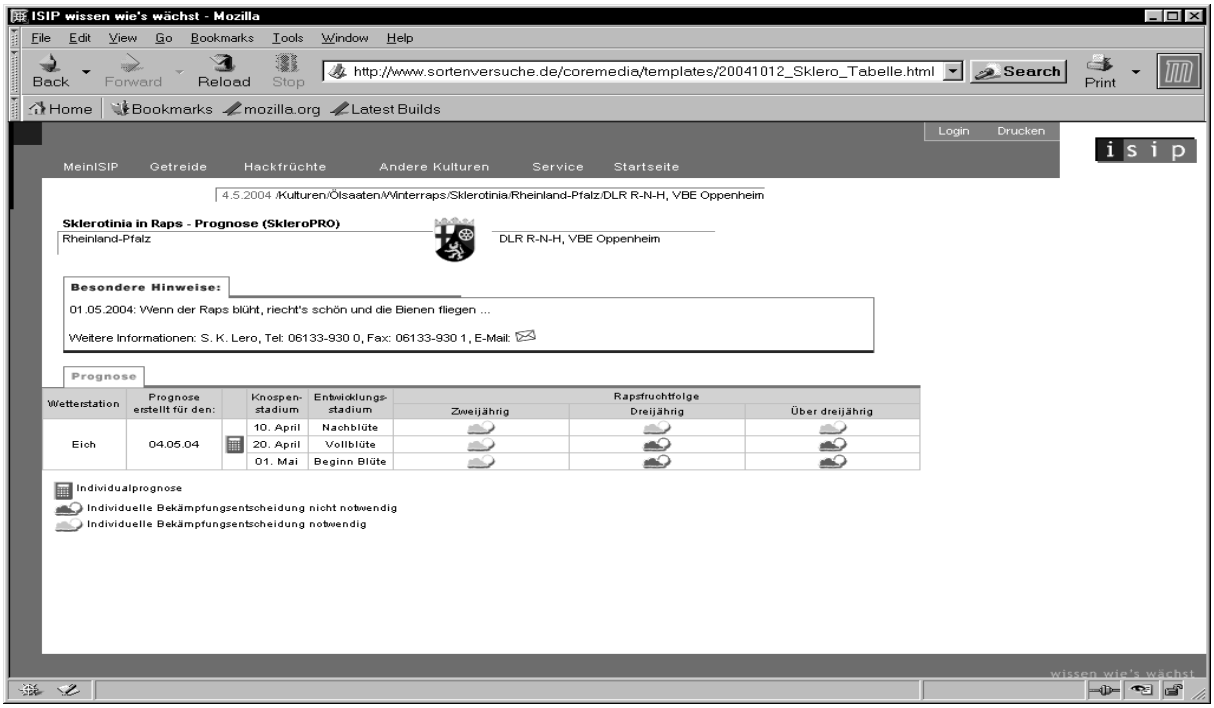


Figure 3. Regional prediction of SkleroPro in ISIP available in spring 2006.

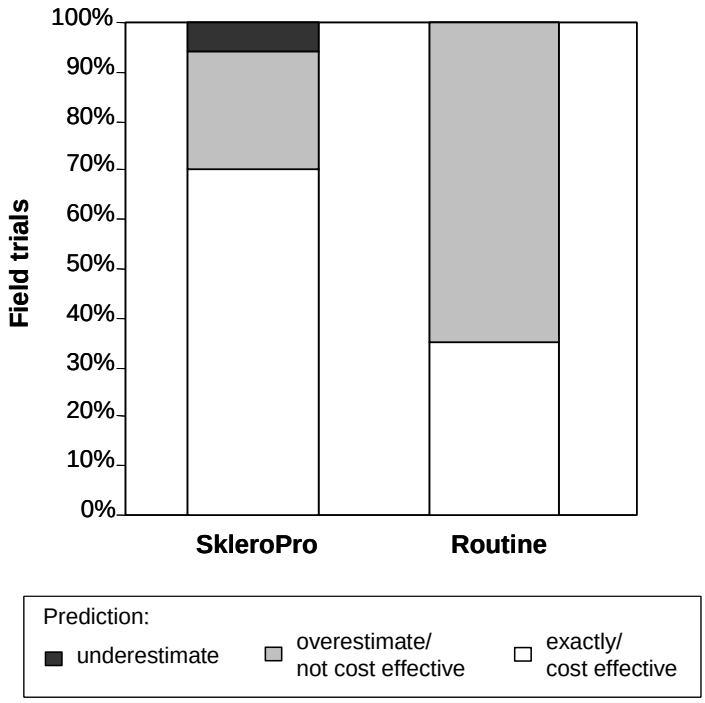


Figure 4. Accuracy of prediction and cost effectiveness of SkleroPro on the basis of 108 field trials done by the state extension services between 1994-2004.

A further examination addressed the precision and economic effects of fungicide timing recommended by SkleroPro compared to a routine spray at full bloom. For this analysis 20 field trials were conducted by the state extension services in 2004 with application dates of treatments either given by the two models and as a routine spray. In these field trials the time

of fungicide application was the only variable. The fungicide Boscalid was used at an application rate of 0.5 l per hectare. Due to precise timing of applications, SkleroPro achieved an average yield gain of more than 0.3 t per hectare in comparison to a routine treatment. This corresponds to an extra profit of more than 13 € per hectare.

Acknowledgement

We gratefully acknowledge the significant input of data and field experiments by the Federal States Plant Protection Services in Germany. This study was supported by grants from the German Federal Ministry of Consumer Protection, Food and Agriculture (BMVEL) and the Federal German Environmental Foundation (DBU). We also thank Isabel Müller and Michaela Meyer for their excellent technical assistance.

References

Abawi, G.S. & Grogan R.G. (1979). Epidemiology of diseases caused by *Sclerotinia* species. – Phytopathology 69: 899-904.

Clarkson, J.P.; Phelps, K.; Whipps, J.M.; Young, C.S.; Smith, J.A. & Watling, M. (2004). Forecasting *Sclerotinia* disease on lettuce: toward developing a prediction model for carpogenic germination of Sclerotia. – Phytopathology 94: 268-279.

Friesland, H. (1998). Ein agrarmeteorologisches Vorhersageverfahren für den Rapskrebs (*Sclerotinia sclerotiorum*) in Winterraps. – Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft 357: 64.

Friesland, H. (2000). Agrarmeteorologisches Verfahren zur Prognose von Rapskrebs. – Raps 18 (1): 14-17.

Krüger, W. (1975). Influence of the weather on attack of rape by *Sclerotinia sclerotiorum* (Lib.) de Bary. – Nachrichtenblatt Deutscher Pflanzenschutzdienst 27: 1-6.

Mueller, D.S.; Hartmann, G.C. & Pedersen, W.L. (2002). Effect of crop rotation and tillage system on sclerotinia stem rot on soybean. – Canadian Journal of Plant Pathology 24: 450-456.

Pope, S.J.; Varney, P.L. & Sweet, J.B. (1989). Susceptibility of cultivars of oilseed rape to *Sclerotinia sclerotiorum* and the effect of infection on yield. – Aspects of Applied Biology 23: 451-456.

Wahmhoff, W. (2000). Integrierter Rapsanbau: Untersuchungen zur Entwicklung integrierter Produktionsverfahren am Beispiel des Winterrapses (*Brassica napus* L.). – Erich Schmidt Verlag.

Walker, A. (1997). ModelMaker, 4.0 edn. – Cherwell Scientific, Oxford, UK.

Young, C.S.; Clarkson, J.P.; Smith, J.A.; Watling, M.; Phelps, K. & Whipps, J.M. (2004). Environmental conditions influencing *Sclerotinia sclerotiorum* infection and disease development in lettuce. – Plant Pathology 53: 387-397.

Weiss, A.; Kerr, E.D. & Steadman J.R. (1980) Temperature and moisture influences on development of white mold disease (*Sclerotinia sclerotiorum*) on great northern beans. – Plant Disease 64 (8): 757-759.

Petal test – Success and disappointments in sclerotinia stem rot forecasting in Poland and China

Małgorzata Jedryczka [1], Piotr Kachlicki [1] and Qiangsheng Li [2]
[1] *Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland,*
[2] *Institute of Crop Research, Anhui Academy of Agricultural Sciences, Hefei, China*

Abstract: Sclerotinia stem rot or white mould, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is one of the most damaging fungal diseases of oilseed rape worldwide. In China, the worlds largest producer of rapeseed, this disease causes considerable yield loss. In Poland, sclerotinia stem rot and stem canker of crucifers are the two most damaging diseases of winter oilseed rape (*Brassica napus* L. forma *biennis*), whereas spring oilseed rape (*B. napus* L. forma *annua*) is mostly infected by black spot and sclerotinia stem rot. Primary sources of plant infection are ascospores produced in apothecia. These fruiting bodies of the perfect stage germinate in the spring on sclerotia, the dormant mycelium formed in black lumps or nodules, inside stems infected with the pathogen. At first, ascospores infect petals, which then fall down onto leaf surfaces and cause disease within the infected plant tissue.

The petal test serves as the basis of all forecasting systems for sclerotinia stem rot on oilseed rape. The test uses selective media with pH indicators, that change the colour of the medium when it is acidified with oxalic acid, secreted by *S. sclerotiorum* during its growth.

We studied discolouration of Steadman's medium supplemented with different pH indicators which change pH in the range 4.5 – 5.5. Based on experiments using mycelial discs obtained from pure cultures of S. sclerotiorum, we have chosen bromophenol blue and bromocresol green as two supplements showing clear and easily assessable results. These two reagents were subsequently used for tests with petals of different cultivars and field situations in China and Poland.

Comparison of results of a petal test and natural infection of oilseed rape with sclerotinia stem rot in Poland

Małgorzata Jedryczka [1], Agata Tekiela [2] and Mariola Glazek [3]
[1] *Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland*
[2] *Regional Experiment Station of the Institute of Plant Protection, Rzeszow, Poland*
[3] *Institute of Plant protection, Branch Sosnicowice, Poland*

Abstract: In spring 2005, petals of oilseed rape (*Brassica napus* L.) were sampled from flowering plants from commercial fields and cultured on BB and BCG media, supplemented with bromophenol blue or bromocresol green pH indicators. Samples were collected 24 times from 19 different fields located in different places throughout Poland. Some fields were treated and some were untreated with fungicides. Each time, a field sample consisted of 30 Petri dishes containing 20 ml of a given medium with 7 petals uniformly placed on each dish. Petals from the same inflorescence were placed on the same Petri dish, with one petal from a randomly chosen flower on BB medium and a petal from the same flower on BCG medium. Petals were placed on a Petri dish in the same order on all plates, which allowed us to study the infection of the same flowers and inflorescences on two media in parallel. Sampling was performed over 20 days in May, with one field sampled 3 times, every nine days. Sampling was also done from four separate replicates of control fields of two experiments using different cultivars in one location. There were also studies to compare the infection of petals of inflorescences located in upper and lower parts of the oilseed rape canopy, as well as studies comparing the infection of different parts of flowers and also studies of infection of leaves and siliques. For comparison, petals were placed on media directly in the field and a few hours later, in laboratory conditions.

In general, upper flowers were more infected than flowers which were collected from lower parts of the canopy. Petals were the most infected parts of flowers. The use of fungicides decreased petal infection by half. Considerable differences were found between the two media on intermediate days of observation (day 3 to day 5), but the final results (obtained on day 4 to day 6) were comparable, with results slightly higher for BCG than for BB medium. The highest infection level of petals was observed at the beginning of the flowering period. There were no considerable differences concerning the final result, irrespective of the place used to put petals on the media. Two different cultivars at one location were infected with the same percentage. At final assessment, the percent infection of petals varied from 19.9% to 90.5%.

Studies on the germination of sclerotia and formation of apothecia of *Sclerotinia sclerotiorum*

Yong Liu[1,2] and Volker H. Paul[1]
[1] *Labor für Biotechnologie und Qualitätssicherung, Fachhochschule Südwestfalen, Soest,*
[2] *Institute of Plant Protection, Sichuan Academy of Agricultural Sciences, 610066, Chengdu, P.R. China,* [*] Corresponding author: e-mail: liuyong65@hotmail.com

Abstract: A method has been developed and optimized to study germination of sclerotia and apothecia formation under continuous temperature conditions. This method allowed differentiation of the origin of 33 different sclerotia isolates and also the behaviour of germination of the origin of *S. sclerotiorum* isolates. Wheat kernels are highly suitable for production of sclerotia. Development of apothecia was optimal under conditions of 10°C and 500 lux and sclerotia germinated after 2-3 months of incubation. Germination of sclerotia and formation of apothecia could be differentiated according to the origin of sclerotia.

In genereal, sclerotia of isolates originally coming from warmer areas germinated more readily. Sclerotia germinated in different substrates, such as 'Einheitserde', sand and vermiculite. Germination of sclerotia which had been developed on especially nutrient rich or nutrient poor media was impaired. One apothecium produced more than 1 million ascospores and was viable more than 15 days at 10°C and 500 lux.

Importance of application mode for the efficacy of CONTANS (*Coniothyrium minitans*) in biocontrol of *Sclerotinia sclerotiorum*

Ibriam Daoud, Katrin Hedke and Andreas von Tiedemann
Georg-August-University of Göttingen, Department of Crop Sciences, Division of Plant Pathology and Plant Protection, Grisebachstrasse 6, D-37077 Göttingen, Germany

Abstract: CONTANS WG®, a biofungicide containing the living conidia of *Coniothyrium minitans* (CM), is registered in Germany for biocontrol of *Sclerotinia sclerotiorum* since 1998. Its main application is in oilseed rape for control of stem rot. Here, we report laboratory and field experiments conducted in order to evaluate the biocontrol efficacy of CM under practice conditions. CM was capable of degrading living sclerotia of *S. sclerotiorum* at soil conditions ranging from 10 to 90% water holding capacity (WHC) and from 1 to 29 °C. Significant reduction in antagonistic activity only occurred at dry conditions below 20% WHC and at soil temperatures below 5 °C. At conditions from 13 to 25 °C and 20 to 90% WHK, sclerotia were degraded within 8 weeks at rates of 90% or more. However, preservation of CM conidia increased at lower soil temperatures. In mini-plots in the field, the antagonist initially applied at a rate of 3.3 x 105 cfu g-1 soil, was still detectable after 72 months at rates sufficient to effectively reduce the survival of sclerotia to levels between 6.6 and 0.2%. The minimum rate of CM in soil for a more than 90% degradation of sclerotia within six months was 1.9 x 103 cfu g-1 soil. In order to optimise the biocontrol efficacy of CM, the timing of application within a rapeseed-cereal crop rotation was varied by either applying CONTANS WG® on the oilseed rape stubble (SR), on the stubble of the previous barley crop (SB) or on the soil prior to sowing of oilseed rape (PS). Appearance of apothecia was recorded in the three following seasons. Three years after application, no apothecia were found in SR, while in SB and PS apothecia still occurred at elevated rates. Further laboratory studies revealed complete inhibition of CM conidia germination in unsterile natural soil, clearly implying a lack of potential of the antagonist to reach sclerotia at any distance apart. Therefore the direct targeting of this antagonist appears essential for realizing its biocontrol potential.

Verticillium

Verticillium wilt on Brassica oilseed crops

Christina Dixelius[1], Ingrid Happstadius[2] and Gunilla Berg[3]
[1] *Department of Plant Biology & Forest Genetics, SLU, Box 7080, 750 07 Uppsala, Sweden, Christina.Dixelius@vbsg.slu.se*
[2] *Svalöf Weibull AB, 268 81 Svalöv, Sweden ingrid.happstadius@swseed.com*
[3] *Swedish Board of Agriculture, Plant Protection Service, Box 12, 230 53 Alnarp, Sweden, gunilla.berg@sjv.se*

Abstract: This presentation on Verticillium wilt in Brassica oilseed crops in Sweden aims to give a historic background to the disease problem as well as to present recent work on both the disease causing organism, its biology and plant breeding actions taken. The severe disease incidence present in Skåne and Östergötland is most likely due to the very intensive cultivation, in some places monoculture, of Brassica oilseed crops between 1945 and 1955. Molecular studies of Verticillium isolates from diseased plants have revealed that *Verticillium longisporum* is the disease causing organism. *V. longisporum* is closely related to *V. dahliae* and *V. albo-atrum* and can easily be confused with *V. dahliae*, especially when considering morphological characters. The overall colonization pattern of *V. longisporum* in oilseed rape seems to be in concordance with earlier reports concerning *V. dahliae* in a range of host species. However, *V. longisporum* preferentially infect members of Brassicaceae. The dispersal, propagation, and long-term survival of this pathogen is mediated through the microsclerotia. Analyses of soil samples from Skåne and Östergötland have revealed high levels of microsclerotia and a presence of *V. longisporum*, *V. dahliae* and *V. tricorpus* in the soil. All *Brassica napus* germplasm is susceptible to Verticillium wilt. Thus, gene bank accessions of both *B. oleracea* and *B. rapa* origin have been evaluated to identify new resistant material. Enhanced levels of resistance were found within both species and these novel genotypes are now being incorporated in *B. napus* breeding programmes. This widening of the gene-pool of *B. napus* will also be of great value to future hybrid breeding programs.

Verticillium wilt in Sweden – Incidence, field scoring and importance

Gunilla Berg[1], Christina Dixelius[2] and Ingrid Happstadius[3]
[1] *Swedish Board of Agriculture, Plant Protection Center, Box 12, 230 53 Alnarp, Sweden, gunilla.berg@sjv.se*
[2] *Department of Plant Biology & Forest Genetics, SLU, Box 7080, 750 07 Uppsala, Sweden christina.dixelius@vbsg.slu.se*
[3] *Svalöf Weibull AB, 268 81 Svalöv, Sweden ingrid.happstadius@swseed.com*

Abstract: The aim of this paper on Verticillium wilt is to show the difficulties with the assessment of the disease. Often plants are infected not only by Verticillium wilt but also with *Phoma lingam*. Therefore, determination of disease severity by visual assessment is uncertain.

Verticillium wilt, caused by *Verticillium longisporum*, is a very serious disease in some regions of Sweden. The problem is mainly due to intensive oilseed cultivation in the early 1950s, sometimes even as a second crop. The high inoculum density of microsclerotia has been maintained by weeds and crop rotation. A crop rotation with four years between every oilseed crops has been practiced since the late 1950s. The yield of winter oilseed rape in Sweden has increased very slow since the 1970s and has varied between 22-36 dt/ha. There are many reasons for this big variation in yield, but Verticillium wilt is definitely one explanation. The impact on yield from Verticillium wilt varies very much between different years. When infected plants show visible microsclerotia and senescence, the yield can be reduced by 30-50 %. But in other years, when only bronze-coloured symptoms appear, the yield is little affected.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 357-360

Determination of risk-factors for the occurrence of *Verticillium longisporum*

Holger Kreye[1], Peter Steinbach[2] and Gerhard Wolf[3]
[1]Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection of Field Crops and Grassland, Messeweg 11/12, 38104 Braunschweig, Germany; [2]Plant Protection Service Mecklenburg Western Pomerania, Graf-Lippe-Str. 1, 18059 Rostock, Germany; [3]Georg-August-Universitiy of Göttingen, Department of Crop Sciences, Division of Plant Pathology and Plant Protection, Grisebachstr. 6, 37077 Göttingen, Germany

Abstract: In a two-year monitoring experiment the occurrence of *Verticillium*-wilt in oilseed rape could be confirmed for all surveyed regions in Germany. However, there are some regional differences in the infestation frequency. In north- and northeast-Germany, traditionally areas where oilseed rape production is highly-intensive, the disease was more frequently diagnosed. The data obtained from the determination of risk-factors showed a clear effect by the percentage of infected oilseed rape during crop rotation with *V. longisporum*. By extending the oilseed rape crop rotation to longer intervals (more than three years) and an additional growing of non-host plants in close cereal/oilseed rape crop rotations, the risk of an infestation by this pathogen could be lowered. In addition, a longer rotation period between oilseed rape crops had a positive effect on subsequent yield. However, the importance of conservation tillage systems as a risk-factor could only be basically confirmed and further investigations are still required. The same conclusions were reached with respect to differences in varietal susceptibility which recently became apparent. There was no effect associated with the application of organic nitrogenous fertilizer, soil type, pH-value and the application of fungicides on the occurence of *Verticillium*-wilt disease.

Key words: *Verticillium*-wilt disease, *Verticillium longisporum*, oilseed rape, crop rotation.

Introduction

Verticillium-wilt has been reported for many years in the traditional oilseed rape cultivation areas in the northern German regions Schleswig-Holstein and Mecklenburg-Vorpommern. The number of reports from other parts of Germany has increased during the end of the 1990's. Therefore, the DPG (Deutsche Phytomedizinische Gesellschaft) committee on oilseed rape carried out a monitoring study for this disease and subsequently an occurrence of the disease in all German regions could be formulated. In addition, the incidence of the infection during the years 2000 to 2003 was observed to increase. However, the locations surveyed were not selected by chance, but were targeted as “suspicious locations” and areas with new variety tests. Higher incidences were determined in Schleswig-Holstein and Mecklenburg-Vorpommern, compared to other regions of Germany (Steinbach *et al*., 2005). These results led to the examination of the reasons for the differences in incidences and to a subsequent research project where monitoring continued for additional 2 years (2002, 2003).

Material and methods

For monitoring, stubble samples of winter oilseed rape (BBCH 92-98) were collected during 2002 and 2003 after harvest from locations throughout Germany. Samples were examined for infection with *Verticillium* sp. by visual assessment and afterwards tested by using an ELISA-test which was developed at the University of Göttingen (Cernusko 1995). Additionally, at each location a soil sample was taken which was examined for the occurrence of micro-sclerotia of *Verticillium* sp. by a Petri-dish-test containing a selective medium (Termorshuisen 1997). The visual assessments were done centrally at the BBA. The ELISA-tests were carried out at the University of Göttingen and the Petri-dish-test was performed at the Centre for Plant protection Service of Mecklenburg-Vorpommern. This test is able to detect the amount of microsclerotia in the soil but it can not differentiate between *Verticillium dahliae* and the oilseed rape pathogen *Verticillium longisporum*. The possibility for estimating the risk of a *Verticillium*-infection in a field was also evaluated. Only locations were selected where a contamination with *V. longisporum* was suspected. Therefore, the frequency of the infection was not a measure for the absolute occurrence in Germany. Beside the potential occurrence of *V. longisporum* locations were chosen that exhibited as normal a crop rotation as possible, and should also permit a comparison between conservation tillage and ploughing. At the surveyed locations, data about the crop rotation, cultivation, fertilisation, seed rate, variety, soil type, pH value and the plant protection measures were collected by means of a questionnaire. In cases where it was possible, a survey of the yield took place to estimate the relevance of the infection with regard to yield.

Results and discussion

The evaluation of the two-year investigation indicated that the disease occurs in all important rapeseed cultivation regions in Germany. However, regional severity is very different and a safe diagnosis of the disease is still a problem for farmers. Although samples were to be sent in from suspicious locations, on average over the years only 58% of the samples exhibited an infestation with *Verticillium*. Apart from the locations with a minimum infestation, which were not covered by the sampling, a certain percentage of locations remained, where an assumed infection was not found. This confirms the statements of Wolf and Weinert (2003) that the infection is often visually misjudged. The highest certainty for proof of this is offered by the ELISA-test. Also, a small percentage of the samples in the project was classified visually being not infected but were recognized afterwards by means of the ELISA as weakly infected.

The evaluation of the questionnaires, the assessments and the tests confirm *Verticillium longisporum* as a pathogen associated with crop rotation. As table 1 shows, the occurrence of infestations increases with a higher frequency of rape cultivation in crop rotation. The decrease of the interval of rapeseed by one year did already cause an increase of the *Verticillium* infestation of around 45% and a duplication of soil inoculum**.** If "non-host plants" were added to the crop rotation, the infestation and the soil inoculum were reduced to a lower level.

Beside the effects of crop rotation, the length of the growing season influenced the occurrence of the disease. An extension of the growing season through earlier sowing, increased levels of soil inoculum**.** Therefore, the visible infestation was increased after a two-week extension to the season. However, the cultivation effect on the occurrence of the disease was weaker than expected. In conservation tillage systems the incidence of infestation was slightly higher than in ploughing systems. The influence of the variety was found to be a

factor, but only weakly. The available data record is not sufficient here, in order to provide solid statements.

The apparent, yet small, differences between varieties offer a starting point for resistance breeding**.** Resistance breeding also seems to be a possible future solution for areas with a high soil inoculum content. For the factor nitrogen only small effects on the infestation were shown. Therefore, not the amount of the N-fertilisation seems to be crucial, but the kind of fertiliser: i.e. a portion of organic nitrogen fertilisation in the entire N-quantity led to a smaller infestation but the soil inoculum increased. Here, further investigations must take place, in order to be able to secure the few existing data. This also includes the influence on the structure of population of *Verticillium* sp. and on the decreased rate of the microsclerotia in the soil. Soil type, pH value and fungicide application did not influence the occurrence of the disease during monitoring.

Yield was negatively affected only as a consequence of a high frequency of rape cultivation within the crop rotation. Since this can be accompanied by the incidence of other rape diseases, the effect cannot be attributed exclusively to *Verticillium* infestation. Therefore, negative yield effects were clearly shown only in those areas, which had an infestation frequency of over 75%. Although the results of the tests are preliminary, we can conclude that oilseed rape has high compensation capabilities, resulting in no detectable yield losses at low up to medium infestation levels. Finally, it remains unclear if yield effects become evident at low to medium infestation levels in case of earlier disease development.

Table 1. Effects of arable farming and crop growing factors on the infestation incidence of Verticillium-wilt (*Verticillium longisporum*) in oilseed rape in Germany.

Factor		influence (%)	
		infestation	Soil inoculum
Oilseed rape in the crop rotation (CR)			
	25 %	100	100
	33 %	145	213
CR: cereals-rape:	75% : 25%	100	100
	66% : 33%	142	234
CR: cereals/potato/ leguminous crops		46	90
Growing period	45 weeks (315 days)	100	100
	+ 1 week	117	214
	+ 2 weeks	184	210
	+ 3 weeks	154	195
Tillage system	ploughing	100	100
	conservation tillage	114	129

References

Cernusko, R. 1995: Entwicklung und Erprobung enzymatischer und immunologischer Nachweismethoden für *Verticillium dahliae* Kleb. im Raps (*Brassica napus* L.). – Diss., Univ. Göttingen.

Steinbach, P.; Kreye, H. & Wolf, G.A. 2005: Bundesweites "Verticillium-Monitoring" Bewertung von Diagnosesicherheit und Risikofaktoren. – Raps 23(1.): 20-26

Termorshuisen, A.J. (1997). Improvement of the assays to detect microsclerotia of *Verticillium dahliae* in soil: Co-operation is necessary. – Presented at the 7th International Verticillium-Symposium, Athens, Greece.

Wolf, G.A. & Weinert, J. 2003: Problemschläge früh erkennen. – DLG-Mitteilungen 7/2003: 48-50.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 361-370

Verticillium longisporum in winter oilseed rape – Impact on plant development and yield

Sarah Dunker, Harald Keunecke and Andreas von Tiedemann
Department of Crop Sciences, Division of Plant Pathology and Crop Protection, Georg-August University Goettingen, Grisebachstrasse 6, D-37077 Goettingen, Germany

Abstract: In the last ten years, an increasing incidence of *Verticillium longisporum* has been observed in the German winter oilseed rape production. The impact of this pathogen on yield and plant morphological parameters, the disease development as well as the reaction of different cultivars is still not known. In field trials done over two-years, the yield effect of *V. longisporum* in relation to disease severity was investigated in plots artificially inoculated with different amounts of infested rapeseed straw. Disease development was investigated on plant samples during the whole growing season using an ELISA test. In agreement with the visual disease assessment in the field, fungal spread in the plants could not be detected before the beginning of maturity. In glasshouse experiments, plants of a susceptible and a moderately susceptible cultivar were inoculated using two different methods (root dip inoculation or microsclerotia inoculation). In contrast to the lack of symptom development in the field, an intense stunting effect was observed in infested plants two weeks after inoculation. Disease severity was higher in the susceptible cultivar and fungal spread was faster and more extensive than in the moderately susceptible cultivar. For plants inoculated with microsclerotia, fungal spread was delayed compared to the root dip inoculation.

Key words: Oilseed rape, crop loss, disease/yield loss relations, disease development, fungal spread, field trials, greenhouse experiments

Introduction

The increasing area of rapeseed cultivation in Germany has caused a significant spread of soil borne diseases. Whereas *Sclerotinia sclerotiorum* is a well known pathogen, *Verticillium longisporum* has only increased in incidence in Germany since the mid eighties. This is particularly the case for the intensive rapeseed growing areas of Germany in Mecklenburg-Western Pomerania and Schleswig-Holstein where disease incidence has increased markedly in the recent years.

The production of microsclerotia, which can survive in the soil more than ten years (Heale & Karapapa, 1999), leads to a build up of soil borne inoculum associated with repeated rapeseed cultivation and represents a long term increased infection risk. Since there are no fungicides or resistant cultivars available to the growers, reducing the frequency of rapeseed within the crop rotation is the only way to control the disease.

Little is known about the effect of *V. longisporum* on yield in oilseed rape. Estimates vary from 10 to 50% yield loss (Daebeler *et al*., 1988; Günzelmann & Paul, 1990; Paul, 2003). Single plant investigations revealed yield losses up to 70% due to *V. longisporum* infection (Wolf & Weinert, 2003; Zeise & Steinbach, 2004). As there are compensatory effects in the field, the results from these single plant investigations can not be transferred to whole plot situations.

Due to the late appearance and the unspecific nature of symptoms, the diagnosis of *V. longisporum* in the field is very difficult. The first symptoms that appear in the field are lamellar brownish discolouration of the stem and half side yellowing of the leaves but these are

not observed before the beginning of ripening. Later on the microsclerotia are produced in diseased plant tissue. In the literature there are contradicting opinions as to the time of infection and the fungal spread in the plant. Whereas Wolf & Weinert (2003) and Steinbach *et al*. (2005) did not detect the pathogen in the diseased plants until the beginning of maturition, Zeise & Seidel (1990) and Zhou *et al*. (2006) observed infections to occur earlier in the growing season.

In field trials done over a two year period, disease/yield loss relations were investigated to evaluate the effect of *V. longisporum* on yield. Parallel to effect on yield, the disease dynamics of *V. longisporum* were investigated during the whole growing season using an ELISA test. As *Verticillium* spp. are known to induce plant morphological changes, e.g. stunting of diseased plants (Isaac, 1957; DeVay & Pullman, 1984, Pegg & Brady, 2002), the effect of *V. longisporum* on shoot length and root parameters were investigated in greenhouse experiments. ELISA investigations were carried out on the glasshouse plants to compare the disease development and the fungal spread of these plants with the results of the field trials. All glasshouse experiments were carried out with a moderately susceptible and a susceptible cultivar, to reveal differences between cultivars with different susceptibilities.

Materials and methods

Field experiments

In the years 2002/03 and 2003/04 field trials were done in Goettingen, Lower Saxony in order to investigate the disease/yield loss relationship for *V. longisporum* in winter oilseed rape. Natural infections were minimized as the field experiments were done in fields where rapeseed had not been grown for at least ten years. Plots were arranged in a Latin square design with four replications and a plot size of 10 m x 2.5 m (25 m²). Each plot was divided into a harvest plot (8 m x 2.5 m) and a sampling plot (2 m x 2.5 m), from which plants for further ELISA-investigations were removed. In 2002/03 the cultivar Prince was grown, whilst in 2003/04 the cultivar was Wotan. Sowing was done on August 23^{rd,} 2002 and 26th of August in 2003 at a seed density of 63 seeds/m². The yield was determined for the whole harvest plot at 9% humidity.

Field inoculation and disease assessment

Inoculation consisted of milled naturally infested oilseed rape straw which was applied to the soil. Different amounts of inoculum material was applied to the plots directly before sowing and incorporated into the upper 5 cm of the soil layer. Apart of a non-inoculated control plot, plots were inoculated with either 300, 600, 900 and 1200 g inoculum material per plot. Disease assessment was done in the laboratory on stubble after harvest. One hundred stubble samples that were equally distributed throughout each of the plots were collected and examined for colonisation with microsclerotia under the epidermis, in the stem pith and in or on the roots using a binocular microscope.

Glasshouse experiments

In the glasshouse experiments, plants of the moderate susceptible cultivar Talent and the susceptible cultivar Falcon were investigated. Plants were sown in a soil mixture of compost, standard soil and sand (3:3:1) and grown in the glasshouse at 18°C with a light/darkness period of 14h/10h for one week. Seedlings were vernalised at 4°C for nine weeks under a light/darkness period of 14h/10h.

Root dip and microsclerotia inoculation

V. longisporum isolates Vl 40 and Vl 43 were grown in 200 ml Czapek-Dox media on a rotary shaker (100 rpm) at 20°C for two weeks. The conidia concentration was determined and

adjusted to 1 x 10^6 conidia/ml using sterile tap water. The conidia suspensions of the isolates Vl 40 and Vl 43 were mixed at a ratio of 1:1 and used as inoculum.

After vernalisation, plants were carefully removed from the soil and the roots were rinsed under tap water to remove adherent soil. Plants were inoculated by dipping the roots into the conidia suspension for 30 minutes. Roots of control plants were dipped into tap water for 30 minutes. After the inoculation, plants were re-planted into 13x13 cm pots in the soil mixture described above and grown in the glasshouse.

Microsclerotia were produced as described by Heppner (1995). 20 ml Czapek Dox medium was inoculated with an agarplug (diameter 5 mm) overgrown with the fungus and incubated for 14 days on a rotary shaker (100 rpm) at 20°C. After the incubation time, the 20 ml conidia suspension was transferred to 225 g of a sterilized oat meal-silica sand mixture (1:14 w/w) with 9% sterile distilled water (v/w) and mixed thoroughly. The mixture was incubated for five weeks at 20°C in darkness.

Microsclerotia concentration was determined by transferring 2 g of the airdried substrate to 5 ml of a 70% saccharose solution. After being thoroughly mixed, the mixture was centrifuged for 10 min at 10°C and 1500 x *g*. The supernatant, with the augmented microsclerotia, was decanted over a filter paper (Schleicher & Schuell) and rinsed with distilled water. The procedure was repeated two times to capture all microsclerotia from the substrate. Subsequently the dry weight of the microsclerotia was determined by drying the microsclerotia on a filter paper, which was previously dried at 105°C for 12 hours and weighed to determine the dry weight, at 105°C for 12 hours. The dried microsclerotia were not used as inoculum in the following experiments.

For the inoculation, the soil in the planting pots was mixed with 0.36 mg microsclerotia/g soil. Control plants were inoculated with an equivalent amount of oatmeal-silica sand mixture without microsclerotia.

Disease assessment and root length measurement

The disease assessment was done 138 dpi using the modified assessment scheme described by Krüger (1989) and Holtschulte (1992) based on the presence of microsclerotia (Table 1).

Table 1. Modified disease assessment scale for *V. longisporum* in winter oilseed rape according to Krüger (1989) and Holtschulte (1992).

Rating scale	Disease character
1	No microsclerotia
2	*Intermediate stage*
3	Few microsclerotia
4	*Intermediate stage*
5	Many microsclerotia
6	*Intermediate stage*
7	Strong colonisation with microsclerotia; plant still living
8	*Intermediate stage*
9	Plant is collapsed and blotched with microsclerotia

The root length, including the hypocotyl, was determined destructively at five assessment times; 28, 54, 81, 101 and 138 dpi, during the vegetation period.

After disease assessment and root length measurement the plants were dried at room temperature and milled, and examined for infestation with *V. longisporum* using the ELISA test as described above.

ELISA investigations

The fungal spread in rapeseed plants from the field and the glasshouse experiments with increasing plant age was investigated using an ELISA test for *Verticillium* spp. in rapeseed, developed in the Institute of Plant Pathology and Plant Protection, University Goettingen (Cernusko, 1995; Cernusko & Wolf, 1997).

Plant samples were taken at regular intervals during the growing season. Adherent soil was removed by washing the plants with tap water. Subsequently the plant samples were airdried and divided into root and shoot and milled. The milled material was extracted in extraction buffer (1:20, w/v) overnight at 4°C on a shaker. 1 ml of the extract was transferred to a reaction tube and centrifuged for 10 min at 13000 rpm. The supernatant was used as the sample in the ELISA test.

Microtiterplates (Nunc, Wiesbaden, Gemany) were coated with 100 µl coating buffer and serum-antibodies (1:1000) and incubated at 4°C over night. The plates were washed three times with washing buffer for 3 min. Afterwards free binding sites were blocked with 200 µl blocking buffer + 0.2% BSA and incubated for one hour at 37°C. After another washing step, 100 µl of sample or protein standard were placed in the plates and incubated for four hours at 37°C. Again the plates were washed three times with washing buffer and the detection was done with 100 µl biotinylized antibodies in PPK/Tween (1:2000) 4°C over night. After three washing steps 100 µl Streptavidin Alkaline Phosphatase (1:10000 in PPK/Tween) was added and incubated at 37°C for one hour. After another washing step 100 µl substrate (1 mg p-Nitrophenyl Phosphate/ml substrate buffer) was filled in the wells and incubated in darkness at room temperature. After 1, 2 and 3 hours the extinction at a wavelength of 405 nm and a reference wavelength of 592 nm was measured using a spectrophotometer (Spectra 2, SLT Laboratories, Crailsheim). Each sample was examined in two replications.

Results and discussion

***Yield effect of* V. longisporum**

The effect of *V. longisporum* on yield in relation to the amount of inoculum was investigated in field trials over a period of two year in artificially contaminated field plots. In both experimental years, different levels of disease incidences were observed with higher disease incidence in the 2002/03 season up to 54.15% in contrast to only 33.75% in 2003/04. In both experimental years disease incidences of 12.04% and 9.25% were detected in the non-inoculated control plots. According to the lower disease incidences in 2003/04, lower amounts of soil borne inoculum could be detected after inoculation using a soil plating technique (data not shown). Positive correlations between disease incidence and amount of soil borne Verticillium inoculum are described by many authors (Ashworth *et al*., 1972; Ashworth *et al*., 1979; Pullman & DeVay, 1982; Nicot & Rouse, 1987; Paplomatas *et al*., 1992; Heppner, 1995; Xiao & Subbarao, 1998). As well as the amount of soil borne inoculum, the cultivar and the climatic conditions could have an influence on the disease incidence.

Although in both years higher disease incidences could be attained with higher amount of inoculum, no significant yield reduction was observed in relation to the control plots (Table 2). In contrast to this result, definite yield reductions were observed in single plant investigations (Zeise & Seidel, 1990; Zeise & Buchmüller, 1997; Zeise & Steinbach, 2004).

As the investigations of Zeise & Seidel (1990) and Zeise & Steinbach (2004) show the damage effect of *V. longisporum* is influenced not only by the disease incidence but also by the disease severity. In both experimental years, the disease severities were very low, even in the heavily inoculated plots (data not shown), which could be the reason for the absence of a yield effect of *V. longisporum*.

Table 2. Disease incidence and yield (in relation to non-inoculated control) in plots artificially inoculated with different amounts of naturally infested rapeseed straw in Goettingen, Germany. Means of four replications; Tukey test, $p \leq 0.05$.

Amount of inoculum [g/plot]	2002/03, cv. Prince		2003/04, cv. Wotan	
	Disease incidence [%]	Yield [%]	Disease incidence [%]	Yield [%]
Control	12.04 a	100.00 a	09.25 a	100.00 a
300	43.53 b	88.45 a	12.25 ab	106.67 a
600	38.10 b	94.64 a	22.25 abc	103.03 a
900	54.15 b	91.29 a	27.75 bc	104.41 a
1200	48.87 b	98.33 a	33.75 c	105.55 a

Disease dynamics of* V. longisporum *in the field and glasshouse

The disease dynamic and the fungal spread of *V. longisporum* in the plants during the whole growing season were investigated using the ELISA test. In plants from the field collected before winter, no infestation could be detected in either root or shoot material. At the first sampling date after winter at beginning of the vegetation period in March, only small amounts of fungal material could be detected on roots but not on shoot material. Comparable to the appearance of the first visible symptoms of *V. longisporum* in the field, a clear detection of *V. longisporum* in root and shoot material was not possible before the beginning of ripening in June, from all plots. At this time, no differences in the amount of fungal material in the plants were obvious between the different inoculation levels. At harvest, at the end of July, clear differences in the amount of fungal material on the plants were observed according to the amount of inoculum (Fig. 1). Only slight differences in the amount of fungal material were observed between roots and shoots.

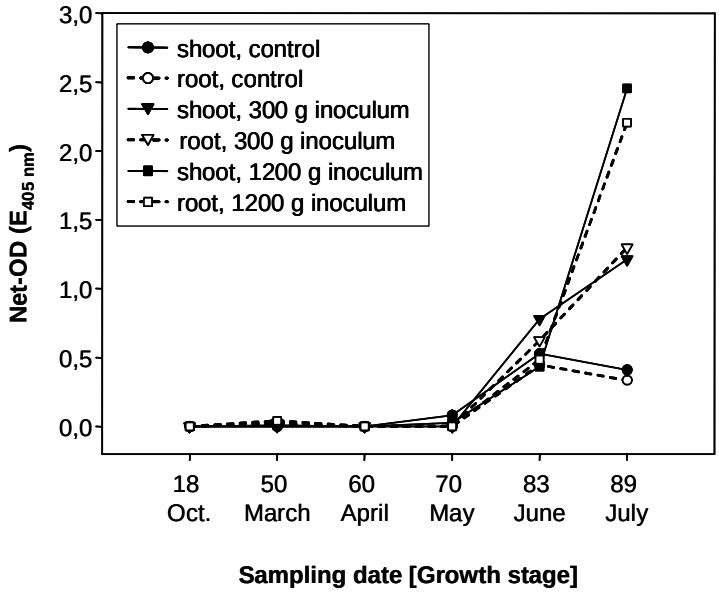


Figure 1. Fungal spread in roots and shoots of plants in the field during the growing season. Means of experimental years 2002/03 (cv. Prince) and 2003/04 (cv. Wotan).

Similar results of the disease dynamics of *V. longisporum* in winter oilseed rape in the field are described by Wolf & Weinert (2003) and Steinbach *et al*. (2005). These investigations also showed that is was only possible to detect the pathogen with the ELISA test at the beginning of ripening at the same time as the first appearance of symptoms on the plants. The detection of small amounts of fungal material in the roots indicates a latent infection that takes place early in the growing season. In experiments of Zeise & Seidel (1990) oilseed rape plants that were re-potted continuously from infected soil to non-infected soil, showed disease symptoms. These results suggested that symptomless *V. longisporum* infection occurred early in the growing season. Several investigations suggested that the change from the vegetative to the generative growth phase influenced the spread of the fungus in the plant and the appearance of symptoms (Zeise & Seidel, 1990; Veronese *et al*., 2003; Zhou *et al*., 2006).

In contrast to the first appearance of *Verticillium* symptoms in the field, symptom development occurred faster on plants in the glasshouse experiments. The comparison of a susceptible (Falcon) and a moderately susceptible (Talent) cultivar indicated that symptom development was slower in the less susceptible cultivar. The first symptoms on leaves (chloroses and blackening of leaf veins) were visible in the susceptible cultivar Falcon after only 28 dpi but were not visible until 54 dpi on the moderately susceptible cultivar Talent.

Considering the fungal spread in the plants investigated with the ELISA test, there were no consistent differences between the two cultivars with respect to the the root dip inoculation method, but there were differences when the microsclerotia inoculation method was used. This inoculation technique delayed fungal spread in roots and shoots on the moderately susceptible cultivar in comparison to the susceptible cultivar (Fig. 2).

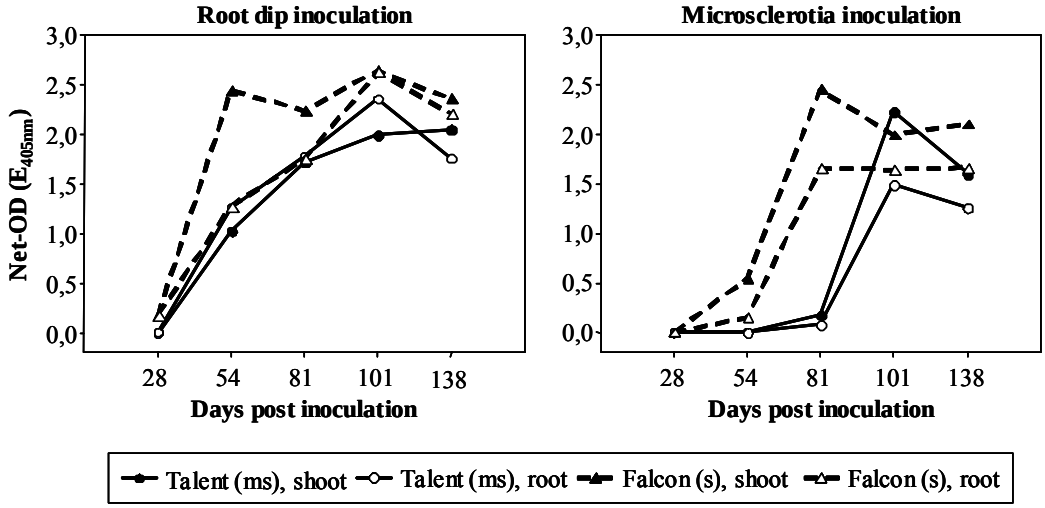


Figure 2. Development of fungal spread in roots and shoots of a susceptible (s, Falcon) and a moderate susceptible (ms, Talent) cultivar after inoculation with conidia (root dip inoculation) or microsclerotia. Means of 15 replications (138 dpi: means of 25 replications).

The comparison of the fungal spread between plants from the field and from the glasshouse is based on the microsclerotia inoculation. This inoculation technique reflects the natural infection mechanism where germinating microsclerotia cause infections. Whereas in the field a clear detection of fungal material was not possible before beginning of ripening, the fungal spread in glasshouse plants began earlier and was clearly detected 54 dpi. Better climatic

conditions for infection and fungal growth could be a reason for the faster fungal spread in the glasshouse plants. Although germination of microsclerotia and mycelial growth is possible at a wide range of temperatures, the optimal conditions are between 15 and 28°C (Pegg & Brady, 2002). These conditions occur in the glasshouse during the whole growing time, whereas the temperature in the field are often below the optimal temperature until the beginning of ripening.

***Effect of cultivar and inoculation technique on disease severity of* V. longisporum**

In the glasshouse experiment, the effect of cultivar and inoculation technique was investigated in the susceptible (Falcon) and the moderately susceptible (Talent) cultivar using two different inoculation techniques. The root dip inoculation was a very intensive inoculation technique with a high probability of effective infection, as the conidia are in direct contact with the roots. This technique is often used in glasshouse experiments to investigate the interaction between *Verticillium* spp. and host plants. The microsclerotial inoculation method reflects the natural infection process. Microsclerotia are mixed in the soil and are not in direct contact with the roots. Infections take place via mycelium from germinated microsclerotia. In this inoculation technique the symptom development starts later because the microsclerotia first have to germinate and the mycelium has to grow towards the roots. In comparison to the fungal spread after the root dip inoculation, a lag phase is observed when using the microsclerotial inoculation method which reflects the germination and mycelium growth (Fig. 2).

According to the more intense inoculation method involving the root dip method, the disease severities achieved were higher than those achieved using the microsclerotial inoculation method (Fig. 3). For both inoculation techniques the susceptible cultivar Falcon showed significantly higher disease severities than the moderately susceptible cultivar Talent.

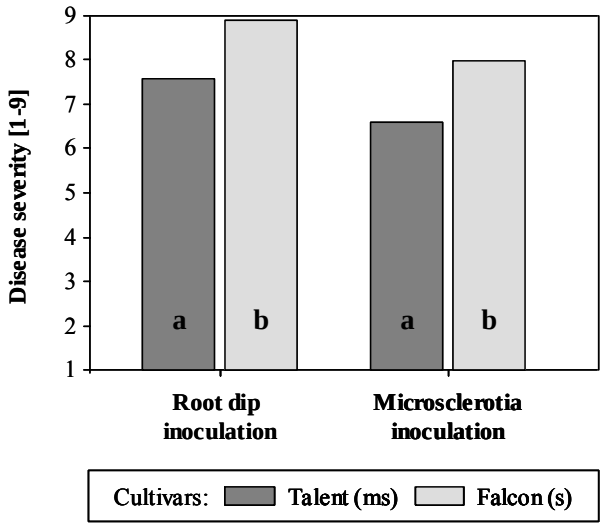


Figure 3. Verticillium disease severity 138 dpi in the greenhouse in a susceptible (s, Falcon) and a moderately susceptible (ms, Talent) cultivar inoculated by root dip inoculation or with microsclerotia. Disease severity: 1 = no disease, 3 = slight, 5 = moderate, 7 = strong, 9 = very strong colonisation with microsclerotia. LSD test, $p \leq 0.05$; means of 25 replications per cultivar and inoculation technique.

Impact of* V. longisporum *on plant development

Especially in glasshouse experiments infections with *Verticillium* spp. lead to plant morphological modifications of the host plants (Pegg & Brady, 2002). For the *V. longisporum* –

oilseed rape system, stunting effects are typical plant morphological symptoms of an infection. These symptoms were not observed in infected plots in the field trials done over a two year period. In our glasshouse experiments, the first stunting effects of *V. longisporum*, in comparison to non-inoculated control plants, were already visible 28 dpi (Fig. 4) in both cultivars and for both inoculation techniques.

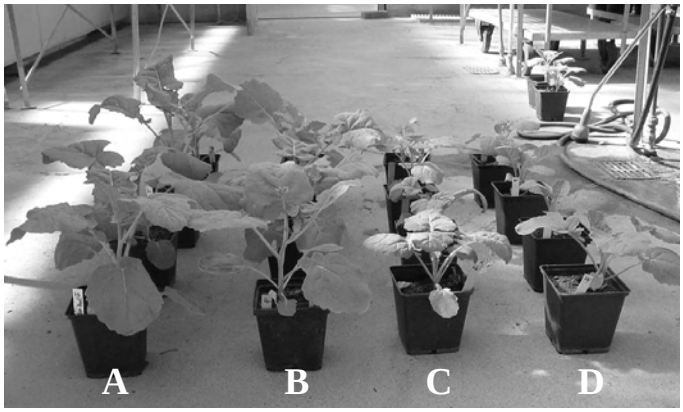


Figure 4. Impact of *V. longisporum* on plant height after artificial inoculation. A: Control plants root dip inoculation, B: control plants microsclerotia inoculation, C: root dip inoculated plants, D: microsclerotia inoculated plants, 28 dpi.

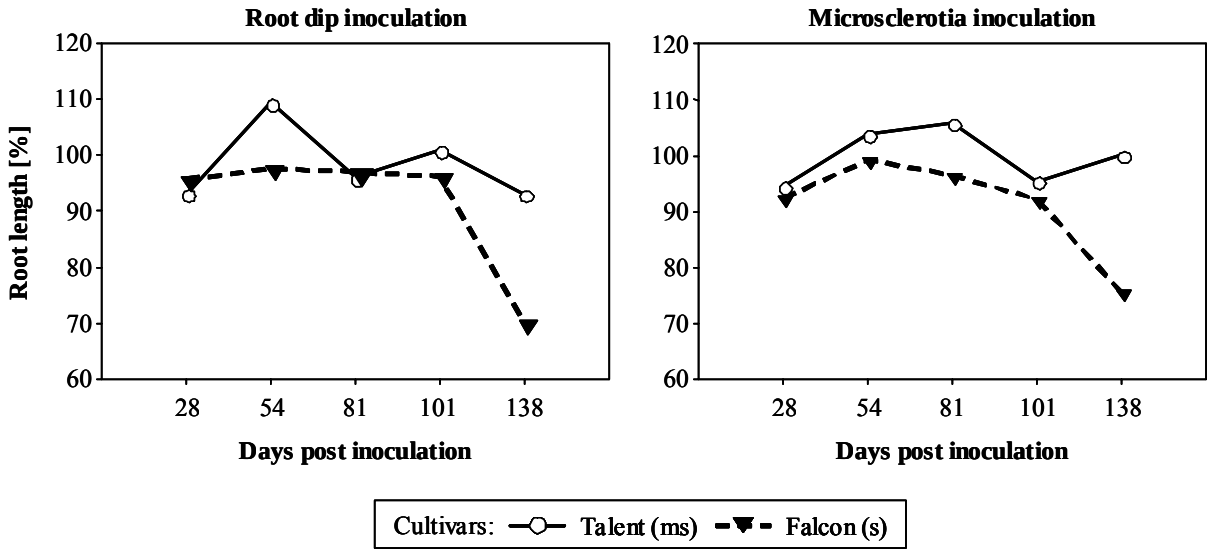


Figure 5. Root length in relation to not inoculated control in plants of a susceptible (s, Falcon) and a moderate susceptible (ms, Talent) cultivar, inoculated with the root dip inoculation and microsclerotia inoculation technique in the greenhouse. Means of 15 replications (138 dpi: means of 25 replications).

As might be expected, a reduction of the shoot dry matter was also observed for the the stunted infected plants. Plants inoculated using the root dip techniques showed a higher reduction in shoot dry matter than plants inoculated with microsclerotia. However, the reduced height of

the susceptible cultivar Falcon was not statistically different to the height of the moderately susceptible cultivar Talent (data not shown).

Besides the impact of *V. longisporum* on shoot parameters, changes in different root parameters due to *Verticillium* infections were investigated. A reduction in root length was not observed for either cultivar or for either inoculation techniques up to 101 dpi. At the last assessment date, 138 dpi, a significant reduction in root length was visible in the susceptible cultivar Falcon whereas the moderately susceptible cultivar Talent showed no reduction in root growth (Fig. 5). Apart from root length, a reduction in root dry matter and the production of lateral roots was observed due to *V. longisporum* infection with greater reductions observed in the susceptible cultivar than in the moderately susceptible cultivar. As was the case for visible symptom development, root dip inoculated plants showed a greater reduction than plants inoculated with microsclerotia (data not shown).

Acknowledgements

We thank Michaela Meier and Hubertus Reintke for excellent assistance in the field. The authors are grateful to Dr. Birger Koopmann and Dr. Neal Evans for improving the manuscript.

References

Ashworth, L.J. JR., Huisman, O.C., Harper, D.M., Stromberg, L.K. & Bassett, D.M. (1979): Verticillium wilt disease of cotton: Influence of inoculum density in the field. – Phytopathology 69: 483-489.

Ashworth, L.J. JR., McCutcheon, O.D. & George, A.G. (1972): *Verticillium albo-atrum*: the quantitative relationship between inoculum density and infection of cotton. – Phytopathology 62: 901-903.

Cernusko, R. (1995): Entwicklung und Erprobung enzymatischer und immunologischer Nachweismethoden für *Verticillium dahliae* Kleb. im Raps (*Brassica napus*). – Dissertation, Georg-August Universität Göttingen.

Cernusko, R. & Wolf, G.A. (1997): Immunological methods for the detection of *Verticillium dahliae* in oilseed rape. – In: Dehne, H.-W., Adam, G., Diekmann, M., Frahm, J., Mauler-Machnik, A., van Halteren, P. (eds.): Diagnosis and identification of plant pathogens. Kluwer Academic Publishers, Dordrecht, Boston, London.

Daebeler, F., Amelung, D. & Zeise, K. (1988): Verticillium-Welke an Winterraps – Auftreten und Bedeutung. – Nachrichtenblatt Pflanzenschutzdienst DDR 42: 71-73.

DeVay, J.E. & Pullman, G.S. (1984): Epidemiology and ecology of diseases caused by *Verticillium* species, with emphasis on Verticillium wilt of cotton. – Phytopathology Medi. 23: 95-108.

Günzelmann, H. & Paul, V.H. (1990): Zum Auftreten und zur Bedeutung der Verticillium-Welke an Raps in der Bundesrepublik Deutschland in 1989. – Raps 8: 23-25.

Heale, J.B. & Karapapa, V.K. (1999): The verticillium threat to Canada´s major oilseed crop: canola. – Canadian Journal of Plant Pathology 21: 1-7.

Heppner, C. (1995): Nachweis von *Verticillium dahliae* Kleb. im Boden mit Plattenguß-verfahren und ELISA (Enzyme-linked immunosorbent assay) sowie Untersuchungen zur Auswirkungen des Inokulums auf den Befall von Winterraps (*Brassica napus* ssp. *oleifera* Metzg.). – Dissertation, Georg-August Universität, Göttingen.

Holtschulte, B. (1992): Untersuchungen zur Biologie und Bedeutung von *Verticillium dahliae* Kleb. und *Leptosphaeria maculans* (Desm.) Ces. et de Not. (Anamorph: *Phoma lingam*

(Tode ex. Fr.)) innerhalb des Erregerkomplexes der Krankhaften Abreife von Raps. – Dissertation, Georg-August University, Goettingen.

Isaac, I. (1957): Verticillium wilt of brussels sprout. – Ann. Appl. Biol. 45: 276-283.

Krüger, W. (1989): Untersuchungen zur Verbreitung von *Verticillium dahliae* Kleb. und anderen Krankheits- und Schaderregern bei Raps in der Bundesrepublik Deutschland. – Nachrichtenbl. Deut. Pflanzenschutzd. 41: 49-56.

Nicot, P.C. & Rouse, D.I. (1987): Relationship between soil inoculum density of *Verticillium dahliae* and systemic colonization of potato stems in commercial fields over time. – Phytopathology 77: 1346-1355.

Paplomatas, E. J., Bassett, D. M., Broome, J. C. & DeVay, J. E. (1992): Incidence of Verticillium wilt and yield losses of cotton cultivars (*Gossypium hirsutum*) based on soil inoculum density of *Verticillium dahliae*. – Phytopathology 82: 1417-1420.

Paul, V.H. (2003): Raps – Krankheiten, Schädlinge, Schadpflanzen, 3. Aufl. – Verlag Th. Mann, Gelsenkirchen-Buer.

Pegg, G.F. & Brady, B.L. (2002): Verticillium wilts. – CABI-Publishing, Oxon, New York.

Pullman, G.S. & DeVay, J.E. (1982): Epidemiology of Verticillium wilt of cotton: A relationship between inoculum density and disease progression. – Phytopathology 72: 549-554.

Steinbach, P., Kreye, H. & Wolf, G.A. (2005): Bundesweites „Verticillium-Monitoring". Bewertung von Diagnosesicherheit und Risikofaktoren. – Raps 23: 20-26.

Veronese, P., Narasimhan, M.L., Stevenson, R.A., Zhu, J.-K., Weller, S.C., Subbarao, K.V. & Bressan, R.A. (2003): Identification of a locus controlling *Verticillium* disease symptom response in *Arabidopsis thaliana*. – The Plant Journal 35: 574-587.

Wolf, G.A. & Weinert, J. (2003): Problemschläge früh erkennen. – DLG-Mitteilungen 7/2003: 48-50.

Xiao, C.L. & Subbarao, K.V. (1998): Relationship between *Verticillium dahliae* inoculum density and wilt incidence, severity, and growth of cauliflower. – Phytopathology 88: 1108-1115.

Zeise, K. & Buchmüller, M. (1997): Studies on the susceptibility to *Verticillium dahliae* Kleb. var. *longisporum* Stark of six related *Brassica* species. – Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 104: 501-505.

Zeise, K. & Seidel, D. (1990): Zur Entwicklung und Schadwirkung der Verticillium-Welkekrankheit am Winterraps. – Raps 8: 20-22.

Zeise, K. & Steinbach, P. (2004): Schwarze Rapswurzeln und der Vormarsch der Verticillium-Rapswelke. – Raps 22: 170-174.

Zhou, L., Hu, Q., Johansson, A. & Dixelius, C. (2006): *Verticillium longisporum* and *V. dahliae*: infection and disease in *Brassica napus*. – Plant Pathology 55: 137-144.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
pp. 371-377

Comparative histological studies on the interaction of *Verticillium longisporum* and *V. dahliae* with roots of *Brassica napus*

Christina Eynck[1], Gisela Grunewaldt-Stöcker[2], Birger Koopmann[1] and Andreas von Tiedemann[1]

[1] *Georg-August-University of Göttingen, Department of Crop Sciences, Division of Plant Pathology and Plant Protection, Grisebachstrasse 6, 37077 Göttingen, Germany*

[2] *University of Hannover, Institiute of Plant Diseases and Plant Protection, Section Plant Pathology, Herrenhäuser Strasse 2, 30419 Hannover*

Absract: *Verticillium longisporum*, the causal agent of Verticillium wilt on oilseed rape (*Brassica napus*) differs from *V. dahliae* in being host-specific to Brassica crops. In order to compare the colonisation and infection processes of these two fungi on roots of *B. napus*, the green fluorescent protein (GFP) was used to label them. Additionally, a conventional fluorescence dye was applied in order to compare the suitability of these techniques for plant-pathogen interaction studies. Using confocal laser scanning microscopy, the colonisation and infection processes were analysed in detail and the following aspects could be observed: (i) the first steps of contact between root and fungi take place at the root hairs, (ii) the preferred colonisation sites on the root surface for *V. longisporum* are the grooves along the junctions of the epidermal cells, (iii) *V. longisporum* does not show a preferation for any specific infection sites, such as wounded tissue or sites of emergence of secondary roots and does not form any specific infection structures, like appressoria. (iv) In contrast to this, *V. dahliae* shows an undirected growth on the root surface and very quickly forms resting structures. Until now, it is not proven wether *V. dahliae* is able to penetrate into the roots or not. Further studies on this non-pathogenic interaction are in progress.

Key words: Verticillium spp., *Brassica napus*, GFP, CLSM

Introduction

Verticillium wilt on oilseed rape (*Brassica napus* L. spp. *oleifera*) is caused by the host-adapted, amphihaploid fungus *Verticillium longisporum* (Karapapa et al., 1997; Zeise & von Tiedemann, 2001, 2002). Because of an increasing area under rapeseed cultivation and the relatively high crop rotation rate, this disease has become a threat to oilseed rape production in Northern Europe (Krüger, 1989; Zielenski & Sadowski, 1995). For both winter and spring rapeseed, breeding for resistance has been severely hampered by the absence of sufficient resistance in available breeding material until recently, promising *B. oleracea* and *B. rapa*-genotypes with enhanced resistance were identified (Happstadius et al., 2003, Dixelius et al., 2005).

Closely related to *V. longisporum* is *V. dahliae*. This ubiquitous soilborne fungus causes wilt diseases on many economically important crops, including cotton, cucurbits, alfalfa, sunflower, eggplant, mint, strawberry, tomato and potato (Bhat & Subbarao, 1999; Domsch et al., 1980; Pegg, 1984; Schnathorst, 1981; Subbarao et al., 1995).

Long-spored *Verticillium* isolates collected from *Brassica* species were used to be classified as *Verticillium dahliae* var. *longisporum* (Stark, 1961) until Karapapa et al. suggested *V. longisporum* to be a distinct species because of several morphological and physiological differences towards *V. dahliae* (Karapapa et al., 1997). However, there is currently controversy concerning the taxonomically classification of *V. longisporum* as a separate, host-

specific species (Fahleson et al., 2004). Thus it has been reported that *Brassica* crops can occasionally host short-spored *Verticillium* isolates (Collins et al., 2003) and that *V. longisporum* is able to infect plants besides of the Brassicas (Fahleson et al., 2003; Johansson et al., 2005). Furthermore, there has been some misidentification of the two species in the last years. Often *V. dahliae* has been regarded to be the causal agent of Verticillium wilt in *Brassica* crops (Söchting & Verreet, 2004, Xiao & Subbarao, 2000).

The host range, epidemiology and infection process of *V. dahliae* have been intensively investigated in previous works (Beckmann, 1987; Gold et al., 1996; Rowe & Powelson, 2002; Schnathorst, 1981). However, little is known about the infection patterns of both *V. longisporum* and *V. dahliae* on roots of *B. napus*. In order to visualize their behaviour in the oilseed rape rhizosphere by confocal laser scanning microscopy (CLSM), we used the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* (Chalfie & Kain, 1998, Tsien, 1998) to label both *V. longisporum* and *V. dahliae*. In addition to the GFP-tagged strains we applied a conventional fluorescence dye in in order to compare these two techniques concerning their suitability for plant-pathogen interaction studies.

Materials and methods

Plant material and fungal isolates

For this study, we chose the winter oilseed rape variety Falcon, supplied by Norddeutsche Pflanzenzucht, Hans-Georg Lembke KG, which is known to be susceptible towards *V. longisporum*. *Verticillium longisporum* isolate VL 43 from *Brassica napus* and *Verticillium dahliae* isolate VD 73 (vegetative compatibility group 2B) from *Linum usitatissimum* were used throughout this work. Both isolates originated from hosts grown in the North of Germany (Zeise & von Tiedemann, 2001; Zeise & von Tiedemann, 2002 a; Zeise & von Tiedemann, 2002 b). Long term storage was performed as conidial suspensions in a concentration of 1-3 x 10^6 conidia mL^{-1} in Czapek-Dox medium supplemented with 25% glycerol at -80°C. For propagation, droplets of the suspensions were plated onto potato dextrose agar (PDA) and incubated for 14 days at 23°C in the dark. Spores were obtained by gently flooding the dishes with 0.9% NaCl solution (modified according to Melouk, 1992).

Bacterial strain

Escherichia coli strain DH5α (Hanahan, 1983) was used during construction and maintenance of plasmids. *Agrobacterium tumefaciens* strain AGL 1 (Lazo, Stein & Ludwig, 1991) was kindly provided by Dr. Susanne Frick, Leibniz Institute of Plant Biochemistry, Halle/Saale. This strain carries the hypervirulent Ti helper plasmid pTiBo542ΔT (Hood et al., 1986; Komari, Halperin & Nester, 1986; Lazo et al., 1991). Short term storage of the *Agrobacterium* cells was performed on solid LB medium supplemented with 25 µg mL^{-1} rifampicin and 50 µg mL^{-1} carbenicillin at 4°C. Cells were stored long term as glycerol cultures in the freezer at –80°C.

Binary vector construction

The binary vector described in this study was constructed on the backbone of pPK2 (Covert et al., 2001). A fragment of about 2.9 kb of gGFP (Maor et al., 1998), containing the SGFP gene driven by the *Aspergillus nidulans* promoter Pgpd and terminated by the *Aspergillus nidulans* trp C terminator (Punt et al., 1987), was isolated by digestion with XbaI and EcoRI.

In two different approaches the plasmid pBS-SK^- (Clontech) was digested on the one hand with the restriction enzymes AflIII and XbaI and on the other hand with AflIII and EcoRI, leading to two fragments of 450 bp and 2.5 kb size, respectively. These fragments were ligated with the XbaI/EcoRI fragment from gGFP in a triparental ligation, resulting in the plasmid pBS::gpd::SGFP. Subsequently, the SGFP expression cassette was removed from

pBS::gpd::SGFP again by digesting with XbaI and HindIII. The resulting fragment was inserted into the corresponding XbaI/HindIII sites of pPK2. The final construct, pPK2::gpd::SGFP, was introduced into *Agrobacterium tumefaciens* by electroporation (Wen-jun & Forde, 1989) at 2.5 kV, 400 ohms and 25 µF.

Agrobacterium- mediated transformation of V. longisporum and V. dahliae

The *Agrobacterium tumefaciens* strain AGL1, containing the binary vector pPK2::gpd::SGFP, was grown at 28°C for 48h in LB- medium supplemented with rifampicin (25 µg mL^{-1}), carbenicillin (50 µg mL^{-1}) and kanamycin (50 µg mL^{-1}). After reaching an optical density of OD_{660} = 0.6 – 0.9 bacterial cells in a 2 ml aliquot were harvested and washed with induction medium (IM, Bundock et al., 1995) supplemented with 200 µm acetosyringone (AS). Subsequently they were diluted in induction medium to OD_{660} = 0.15. The cells were grown for an additional 6 – 12 h before they were mixed with an equal volume of a spore suspension of either *Verticillium longisporum* isolate VL 43 or *Verticillium dahliae* isolate VD 73 (1 – 3 x 10^6 spores mL^{-1}). From this mixture aliquots of 200 µL were plated on a cellophane membrane placed on solid cocultivation medium (same composition than IM except that it contains 5 mM glucose instead of 10 mM glucose) supplemented with 200 µM acetosyringone. After a cocultivation at 23°C for 60h the already grown fungal mycelium and the *Agrobacteria* were washed away with an 0.9% NaCl solution supplemented with 200 µM cefotaxim (for counterselection against *Agrobacterium tumefaciens*) and plated on Czapek Dox medium containing hygromycin B (50 µg mL^{-1}) as a selection agent for the transformed fungi and again cefotaxim (200 µM). The plates were incubated at 23°C and after 8 – 10 days discrete colonies emerged. Each colony was checked for fluorescence under the fluorescence microscope and those showing the typical GFP fluorescence were subcultured for further studies.

Plant growth conditions and inoculation methods for microscopic examinations

In vitro plant growth and infection

Sterilised seeds of *Brassica napus* cv. Falcon were sown on a cellophane membrane placed on water agar in Petri dishes, preventing the roots from growing into the medium and therefore guaranteeing an undisturbed analysis of the interaction between plant root and fungi. The Petri dishes were subjected to a lighting regime of 14/10 h (light/dark) and a temperature regime of 23/20°C (day/night) in a controlled environment cabinet. After the plants developed a well-defined root system, droplets of a spore suspension of either the transformed *V. longisporum* or the transformed *V. dahliae* strain were placed on the cellophane membrane close to the roots.

Gnotobiotic sand system

Sterilised seeds of *Brassica napus* cv. Falcon were sown in double-autoclaved silica sand. After 3- 4 days the oilseed rape seedlings appeared. In order to prevent damaging of the roots, we avoided to transfer the plants into another substrate. The plants were watered daily and fed two times a week with a full nutrient solution (“Flory Basisdünger”, EUFLOR). The growth temperature in the controlled environment chamber was 23°C in the day (14 h) and 20°C in the night (10 h). A week after germination, the plantlets were inoculated by pouring a spore suspension in a concentration of 1* 10^6 spores mL^{-1} of the transformed *V. longisporum* or *V. dahliae* spore suspension per plant onto the sand.

Microscopy

Microscopic analysis was performed with a Leica TCS SP Confocal Laser Scanning Microscope (Leica, Mannheim, Germany). Digital images were acquired by scanning with optimal settings for GFP or acid fuchsine, respectively.

Results and discussion

To our knowledge, this is the first report on *Agrobacterium tumefaciens* mediated transformation of the phytopathogenic fungi *V. longisporum* and *V. dahliae*, resulting in the stable expression of GFP. The transformants we obtained were indistinguishable from the wild type strain concerning colony morphology, growth rate and pathogenicity on the prevailing host plant. In our case, due to the fact that GFP is constitutively expressed in the cytoplasm of the transformed fungi, only younger hyphae were glowing and thus visible under the fluorescence microscope. Therefore it was not possible to analyse all stages of colonisation and infection processes at one time point under consideration. The phenomenon that older hyphae show a reduced or no expression of GFP has already been described by Eckert *et al*. (2005) in their studies on *Leptosphaeria* spp. and *Oculimacula* spp. In our studies, we directly compared images resulting on the one hand from pure GFP fluorescence and on the other hand from staining with a fluorescence dye. These analyses revealed that not only hyphae in their whole length but also more hyphae could be observed with the classical staining. The unspecific staining of the plant background made it possible to even localise the fungal hyphae in the host tissue on cellular level. Although there are nevertheless quite a few influential arguments for the use of GFP in plant-fungus interaction studies, the good quality of the images resulting from the staining convinced us of the merits of GFP in this case.

Many aspects of our microscopic observations of the interaction of *V. longisporum* with roots of *B. napus* are in agreement with earlier works concerning the infection processes of *V. dahliae* on a wide range of host plants (Beckmann, 1987; Gold *et al*., 1996; Rowe & Powelson, 2002; Schnathorst, 1981). However, our studies provide new information about colonisation and infection processes not described so far. Especially very early interaction events, including the recognition process between host and pathogen in the beginning of disease development could be described. Thus we could observe that the fungus initially approaches the root via the root hairs. Hyphae that were found near the main and lateral roots were intensively interwoven with the root hairs. A tight attachment of the hyphae to the root hairs could be documented at random positions along the root. The root tip was the only part of the root that was not colonised. Already at this stage of interaction, *V. dahliae* colonised the root to a much lesser extend than *V. longisporum* did. In the *in vitro* system, *V. dahliae* produced ample masses of spores among the root hairs as early as 24h after infection. We never observed this for *V. longisporum* throughout the whole time of our investigations.

After approaching via the root hairs, hyphae of *V. longisporum* attach to the root surface where they prefer to grow along the grooves of the junctions of the epidermal cells forming a network of hyphae. Primary infection occurred either at the junctions of epidermal root cells or directly into cells. We could never observe penetration of the root tip or of root hairs. This is in contrast to other studies (Zhou *et al*., 2005) where infection by *V. longisporum* was primarily initiated from lateral roots or root hairs. Our studies revealed that secondary roots are infected either at the site of emergence or at random positions. Furthermore, wounds were not necessary for infection since the fungus is able to penetrate the cells directly. Our findings are similar to the results of Lagopodi *et al*. (2001) who used GFP-expressing *Fusarium oxysporum* f. sp. *radicis-lycopersici* to study tomato root colonization and infection and to those of Oren *et al*. (2002) studying early events in the *Fusarium verticillioides*-maize interaction. Thus, there seems to be a common mode of plant colonization at early interaction stages, in which the pathogens first attach to the root hairs and then penetrate directly into the epidermal cells (Oren *et al*., 2002).

The subsequent spread of the fungus in the root cortex took place by intracellular as well as intercellular growth. Instead of intensively colonising the root cortex, the fungus grew

towards the central cylinder in a more or less directed manner. Remarkably, the roots, although intensively colonised by *V. longisporum*, did not show any symptoms, like discoloration or necrotic lesions. And even the host cells that were occupied by intracellular growing hyphae revealed an intact structure of the cytoplasm.

The interaction of *V. dahliae* with roots of *B. napus* is characterised by a completely different behaviour of the fungus. The undirected growth of hyphae that are only loosely attached to the root surface and the extremely quick production of conidia and microsclerotia outside the root tissue lead to the assumption that *V. dahliae* does not colonise the root tissue of oilseed rape at all. Nevertheless studies on this issue will be continued.

Investigations of oilseed rape plants inoculated with *V. longisporum* revealed a first colonisation of the xylem vessels three weeks after inoculation. The distribution of the fungus was restricted to scattered vessels filled up by mycelium. Adjacent xylem vessels were easily colonised through plasmodesmata. This has already been described for *V. longisporum* (Zhou *et al*., 2005) and *V. albo-atrum* (Heinz *et al*., 1998), but not for *V. dahliae* so far. Spores were formed at the end of phialides that were arranged in a typical verticilliate manner.

In conclusion, our results show, that *B. napus* is either no host for *V. dahliae* or less susceptible towards an infection, as suggested by Zhou *et al*. (2005). This finding might be of importance for oilseed rape growers because crops like potato or sugar beet, that are hosts of *V. dahliae*, are often grown in the same areas (Pegg & Brady, 2002), which implies that microsclerotia from both species can exist in the same soil (Zhou *et al*., 2005).

Acknowledgements

We are grateful for technical assistance by Jutta Schaper. This work is supported by the Federal Ministry of Consumer Protection, Nutrition and Agriculture, Agency of Renewable Resources and the Association of German Plant Breeders (GFP).

References

Beckmann, C.H. 1987: The nature of wilt diseases of plants. St. Paul, MN, USA: APS Press

Bhat, R.G. & Subbarao, K.V. 1999: Host range specificity in *Verticillium dahliae*. Phytopathology 89: 1218-1225

Bundock, P., den Dulk-Ras, A., Beijersbergen, A., and Hoykaas, P.J.J. 1995: Trans-kingdom T-DNA transfer from *Agrobacterium tumefaciens* to *Saccharomyces cerevisiae*. Eur. Mol. Biol. Organ. 14: 3206-3214

Chalfie, M., & Kain, S. 1998: Green fluorescent protein. properties, applications and protocols. Wiley-Liss, Inc., New York

Covert, S.F., Kapoor, P., Lee, M, Briley, A., Nairn, C.J. 2001: *Agrobacterium tumefaciens*-mediated transformation of *Fusarium circinatum*. Mycol. Res. 105: 259-264

Dixelius, C., Happstadius, I., Berg, G. 2005: Verticillium wilt on *Brassica* oil crops – a swedish perspective. Journal of the Swedish Seed Association 115, 36-48.

Domsch, K.H., Gams, W, Anderson, T.-H. 1980: *Nectria* (Fr) 1849, *Verticillium* Nees ex Link 1824. In: Compendium of Soil Fungi, Vol 1, 829-845. Academic Press, New York.

Eckert, M., Maguire, K., Urban, M., Foster, S., Fitt, B., Lucas, J., Hammond-Kosack, K. 2005: *Agrobacterium tumefaciens*-mediated transformation of *Leptosphaeria* spp. and *Oculimacula* spp. with the reef coral gene *DsRed* and the jellyfish gene *gfp*. FEMS Microbiology Letters 253: 67-74

Fahleson, J., Lagercrantz, U., Hu, Q., Steventon, L.A., Dixelius, C. 2003: Estimation of genetic variation among Verticillium isolates using AFLP analysis. European Journal of Plant Pathology 109: 361-371

Gold, J., Lee, B., Robb, J. 1996: Colonization of tomatoes by *Verticillium dahliae*: determinative phase II. Can. J. of Bot. 74: 1279-1288

Hanahan, J. 1983: Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. 166: 557-580

Happstadius, I., Ljunberg, A., Kristiansson, B., Dixelius, C. 2003: Identification of *Brassica oleracea* germplasm with improved resistance to Verticillium wilt. Plant Breeding 122, 30-34

Hood, E.E., Helmer, G.L. Fraley, R.T. & Chilton, M.D. 1986: The hypervirulence of *Agrobacterium tumefaciens* A281 is encoded in a region of pTiBo542 outside of T-DNA. Journal of Bacteriology 168: 1291-1301

Johannson, A., Goud, J.-K., Dixelius, C. 2005: Plant host range of *Verticillium longisporum* and microsclerotia density in Swedish soils. European Journal of Plant Pathology, in press.

Karapapa, V.K., Bainbridge, B.W., Heale, J.B. 1997: Morphological and molecular characterisation of *Verticillium longisporum* comb. nov., pathogenic to oilseed rape. Mycol. Res. 101: 1281- 1294

Komari, T., Halperin, W. & Nester, E.W. 1986: Physical and functional map of supervirulent *Agrobacterium tumefaciens* tumor-inducing plasmid pTiBo542. Journal of Bacteriology 166: 88-94

Krüger, W. 1989: Untersuchungen zur Verbreitung von *Verticillium dahliae* Kleb. und anderen Krankheits- und Schaderregern bei Raps in der Bundesrepublik Deutschland. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 41: 49-56

Lagopodi, A.L., Ram, A.F.J., Lamers, G.E.M., Punt, P.J. 2001: Novel aspects of tomato root colonization and infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. Molecular Plant-Microbe Interactions 15: 172-179

Lagopodi, A.L., Ram, A.F.J., Lamers, G.E.M., Punt, P.J., van den Hondel, C.A.M.J.J., Lugtenberg, B.J.J., Bloemberg, G.V. 2002: Novel aspects of tomato root colonization and infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. MPMI 15: 172-179

Lazo, G.R., Stein, P.A., Ludwig, R.A. 1991: A DNA transformation–competent *Arabidopsis* genomic library in *Agrobacterium*. BIO/TECHNOLOGY 9: 963-967

Maor, R., Puyesky, M., Horwitz, B.A., Sharon, A. 1998: Use of green fluorescent protein (GFP) for studying development and fungal-plant interaction in *Cochliobolus heterostrophus*. Mycol. Res. 102: 491-496

Melouk, H.A. 1992: *Verticillium*. In: Singleton, L.L., Mihail, J.D., Rush, C.M., eds. Methods for research on soilborne pathogenic fungi. St Paul, MN, USA: APS Press, 175-178

Mol, L., Scholte, K. 1995: Formation of microsclerotia of *Verticillium dahliae* Kleb. on various plant parts of two potato cultivars. Potato Research, 38: 143-150

Neumann, M.J., Dobinson, K.F. 2003: Sequence tag analysis of gene expression during pathogenic growth and microsclerotia development in the vascular wilt pathogen *Verticillium dahliae*. Fungal Genetics and Biology 38: 54-62

Oren, L., Ezrati, S., Cohen, D., Sharon, A. 2003: Early events in the *Fusarium verticillioides*-maize interaction characterized by using a green fluorescent protein-expressing transgenic isolate. Applied and Environmental Microbiology 69: 1695-1701

Pegg, G.F. 1984: Life in a black hole – the micro-environment of the vascular pathogen. Trans. Br. Mycol. Soc. 85: 1-20

Pegg, G.F. 1984: The impact of Verticillium diseases in agriculture. Phytopathol. Mediterr. 23: 176-192

Pegg, G.F., Brady, B.L., eds. 2002: Hosts. In: Verticillium Wilts. Wallingford, UK: CAB Publishing, 193-340

Punt, P.J., Oliver, R., Dingemanse, M.A., Pouwels, P.H., van den Hondel, C.A.M.J.J. 1987: Transformation of Aspergillus based on the Hygromycin B resistance marker from *Escherichia coli*. Gene 56: 117-124

Schnathorst, W.C. 1981: Life cycle and epidemiology of *Verticillium*.. In: Mace, M.E., Belt, A.A. & Beckmann, C.H. (eds.), 81-111. Fungal Wilt Diseases of Plants. Academic Press, Inc., New York.

Söchting, H.P., Verreet, J.-A. 2004: Effects of different cultivation systems (soil management, nitrogen fertilization) on the epidemics of fungal diseases in oilseed rape (*Brassica napus* L. var. *napus*). Journal of Plant Diseases and Protection 111: 1-29

Stark, C. 1961: Das Auftreten der Verticillium- Tracheomykosen in Hamburger Gartenbau-Kulturen. Gartenbauwissenschaft 26: 493-528

Subbarao, K.V., Chassot, A., Gordon, T.R., Hubbard, J.C., Bonello, P., Mulin, R., Okamoto, D., Davis, R.M. & Koike, S.T. 1995: Host range of *Verticillium dahliae* from cauliflower and genetic relationships and cross pathogenicities of isolates from different crops. Phytopathology 85: 1105-1112

Tsien, R.Y. 1998: The green fluorescent protein. Annu. Rev. Biochem. 67: 509-544

Wen-jun, S. & Forde, B.G. 1989: Efficient transformation of *Agrobacterium* spp. by high voltage electroporation. Nucleic Acid Research 17: 8385

Xiao, C.L., Subbarao, K.V. 2000: Effects of irrigation and *Verticillium dahliae* on cauliflower root and shoot growth dynamics. Phytopathology 90: 995-1004

Zeise, K., von Tiedemann, A. 2001: Morphological and physiological differentiation among vegetative compatibility groups of *Verticillium dahliae* in relation to *V. longisporum*. J. Phytopathology 149: 469-475

Zeise, K., von Tiedemann, A. 2002: Application of RAPD-PCR for virulence type analysis within *Verticillium dahliae* and *Verticillium longisporum*. J. Phytopathology 150: 557-563

Zeise, K., von Tiedemann, A. 2002: Host specialization among vegetative compatibility groups of *Verticillium dahliae* in relation to *Verticillium longisporum*. J. Phytopathology 150: 112-119

Zhou, L., Hu, Q., Johannson, A., Dixelius, C. 2005: *Verticillium longisporum* and *Verticillium dahliae*: infection and disease in *Brassica napus*. Plant Pathology 54, in press

Zielenski, D. & Sadowski, C. 1995: A preliminary study on *Verticllium dahliae* Kleb. in winter oilseed rape in Poland. In: D. Murphy (ed.), 649-651. Proceedings of the 9th International Rapeseed Conference, Cambridge. 4-7 July 1995. GciRC, Cambridge, UK.

Analysis of systemic signals in the xylem of *Brassica napus* infected with *Verticillium longisporum*

Nadine Riediger[1], Birger Koopmann[1], Andres Schützendübel[1], Petr Karlovsky[1], Ursula Hettwer[1], Derek Marsh[2], Brigitta Angerstein[2] and Andreas von Tiedemann[1]
[1] University of Göttingen, Department of Crop Sciences, Division of Plant Pathology and Plant Protection, Germany. [2]Max Planck Institute for Biophysical Chemistry (Karl Friedrich Bonhoeffer Institute), Am Faßberg 11, 37077 Göttingen

Abstract: *Verticillium longisporum* (VL) is a soilborne, pathogen with host specificity on the Brassicas. Typical symptoms on oilseed rape are premature ripening and stunting under greenhouse conditions. Compared to other Verticillium diseases no wilting occurs on crucifers. There are signalling molecules assumed to cause disease symptoms by altering the developmental physiology of the host plant. A likely candidate is nitric oxide (NO). NO is reported to induce various physiological alterations, such as de-etiolation, inhibition of hypocotyl growth and ethylene production. Therefore, we examined the induction of stunting by application of the NO donor sodium nitroprusside (SNP). SNP, applied over a period of 28 days (three times per week) at different concentrations (10, 50, 100 µM), induced stunting on 'rapid cycling rape' in the climate chamber. Plant growth was reduced in each SNP treated variant and plants were smaller than the ones infected with VL, with the greatest growth reduction at 10 µM SNP. However, after 30 days of growth a difference in shoot length was no longer detectable. At present, it is unclear whether the nitric oxide level in the plant is (i) up- or down-regulated during infection with the vascular pathogen, (ii) whether the pathogen produces NO scavenging proteins, or (iii) whether VL directly alters the NO metabolism of the plant so that NO is no longer produced. First results indicate that the NO level in VL infected plants is lower than in uninfected plants. As NO is known to delay senescence, a lower NO level may result in premature flowering, an effect observed on field-grown oilseed rape upon infection with VL. Hence the physiological effects of VL infection and NO application were analysed with relation to NO levels in plant tissue. Therefore, we developed an indirect NO analytical method by using an NO specific scavenger, carboxy-PTIO, which reacts with NO in a stoichiometric manner. NO can be detected by measuring the specific reaction product (cPTI) with HPLC mass spectrometry. We extracted xylem sap from infected plants with a pressure bomb and used the samples for the quantitative analysis of NO. Alternatively, measurements were conducted with electron-resonance-spectroscopy (ESR). Here, NO is scavenged and stabilised by 'spin trap' molecules and measured due to its properties as a free electron radical, by recording the changes in a magnetic field, which creates specific signal peaks. Since NO can derive from different sources e.g. nitrate/nitrite reductase or a presumed NO synthase, the level of nitrite in infected and uninfected plants in relation to NO levels was recorded. In a further analytical approach NO is indirectly determined by using a nitrite specific fluorescence dye diaminonaphthalene (DAN), whose derivate NAT can be detected by fluorescence- HPLC.

Other diseases

Consequences of oilseed rape infection with phytoplasma like organisms

Michał Starzycki[1], Eligia Starzycka[1] and Jan Pszczoła[2]
[1] *Plant Breeding and Acclimatization Institute, Department of Oil Crops, ul. Strzeszyńska 36, 60-327 Poznań, Poland, e-mail: michals@nico.ihar.poznan.pl, fax +48 (61) 8233871*
[2] *Borowo Department of the Plant Breeding Company Strzelce Ltd., Borowo, 64-020 Czempin, Poland, e-mail: janpszczola@pf.pl, fax +48 (61) 28 26 297*

Abstract: On winter oilseed rape plantations in Poland and in other countries, some "shaggy" looking plants can sporadically be observed. These deformations are usually caused by phytoplasmas, that settle the sieve tubes (phloem) of affected plants. Morphological changes of inflorescences and single flowers was described for the first time by Schmidt (1955). In the following years other authors: Lehmann (1969), Horvath (1969), Gundersen et al. (1994) described the etiology and symptomatology of the pathogen which was responsible for growth aberration and irregular organogenesis. Initially, it was thought that yellow type viruses were responsible for such a situation (Valenta & Musil 1963). But further investigations excluded this hypothesis and pointed at mycoplasma-like organisms as the actual perpetrators (Sears & Kirkpatrick 1994, Gundersen et al., 1994). To distinguish them from bacterial animal pathogens, so called mycoplasms, bacteria that settle on plants was termed "phytoplasmas". The vectors of phytoplasmas are insects of *Jasside* family. In the insect the pathogen occurs as inclusions. At present the identification of possible phytoplasmas and their pathotypes is possible with the use of molecular techniques.

Isolation of total DNA was done by the Doyle and Doyle method (1990). For PCR analysis two pairs of universal primers for identification of *Phytoplasma* were used: rU3/fU5 that amplifies about 880 bp (Lorenz et al., 1995) and rA16/fA16 (Ahrens & Seemüller 1992, Schneider et al. 1993) that amplifies 558 bp. As a standard of phytoplasma from group AAY (Kamińska & Korbin, 1999) DNA of infected plant *Catharanthus roseus* L. was used (Kamińska et al., 1996).

Additionally from some of less infected plants seeds were received, which were sown again. After the vernalisation period, only "shaggy" plants were isolated in the flowering phase and put to haploidysation (Cegielska-Taras & Szała 1997). Observations of haploids development were made from seedling stage (*in vitro*) to grown-up stage (*in vivo*). The same treatment was applied to diploid plants received from seeds that were descendent from self-pollinated "shaggy" plants.

Effect of temperature on development of lesions caused by *Alternaria* spp. on leaves of oilseed rape

Aneta Gutowska [1], Paulina Borowczak [1] and Małgorzata Jędryczka [2]
[1] *Agriculture University of Poznan, Poland*
[2] *Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland*

Abstract: Leaves, stems, pods and seeds of oilseed rape (*Brassica napus* L.) can be infected by species belonging to the genus *Alternaria*. Pathogenic species are responsible for black spot disease which can cause considerable problems, especially in cases of pod infection before harvest. Heavy infection of pods may result in poor quality of seeds used for processing, poor storage and low quality of sowing material. In Poland, black spot is regarded as an important problem in cultivation of both spring and winter oilseed rape.

In our experiment we have used 12 isolates belonging to six following species: *A. brassicae* (3 isolates), *A. brassicicola* (3), *A. alternata* (3), *A. radicina* (1), *A. porri* (1) and *A. dauci* (1). The aim of this experiment was to compare development of disease symptoms on leaves of oilseed rape at different temperature regimes. Experiments were performed in a controlled environment at 16°C, 18°C and 22°C. Fungal isolates were cultured on SNA medium, that allowed to form numerous spores. Symptom development was studied on the third and fourth leaves of cv. Bosman. Leaves were cut and placed in plastic containers, with petioles wrapped up in filter paper soaked with distilled water. Special supports prevented contact of leaf surfaces with the wet paper. Leaves were inoculated with 6 mm diameter agar discs placed in the middle of each half of a leaf. Each isolate was placed on intact leaves and on leaves wounded with a needle. Each variant (isolate x temperature x leaf treatment) had twelve replicates. Experiments were run for two weeks. Symptoms resulting from inoculation were scored every three days. Discolouration of a leaf was measured in two perpendicular directions: along the side vein and across the leaf. Measurement of symptoms on leaf was done separately for black, yellow and light green zone.

The largest symptoms on leaves were caused by *A. brassicicola* with the mean size of the whole leaf symptom reaching 29.5 mm for wounded leaves and 22.4 mm for intact leaves (observation 11 days after treatment). Symptoms caused by *A. brassicae* were about half the size of symptoms caused by *A. brassicicola*. Symptoms produced by other species vere negligible or non existent when the leaf surface was not wounded prior to depositing of an inoculum. Symptom development greatly depended on temperature, with the smallest spot sizes at 16°C and the largest symptoms at 22°C. Wounding speeded up the infection process and resulted in large symptoms on leaves.

Integrated Control in Oilseed Crops
IOBC/wprs Bulletin Vol. 29(7) 2006
p. 387

The incidence of *Alternaria* spp. on seeds of chosen population and hybrid oilseed rape cultivars

Ewa Jajor and Marek Korbas
Institute of Plant Protection, Department of Mycology, Miczurina 20, 60 - 318 Poznań, Poland

Abstract: Often in Poland, on leaves, stems and siliques of oilseed rape plants, symptoms of black spot are observed. The casual agents of black spot are fungi of the genus Alternaria, mainly *Alternaria brassicae, Alternaria brassicicola* and *Alternaria alternata*. Often from diseased siliques diseased seeds are obtained. Spores and mycelium of *Alternaria* spp. are present on the seed surface or the mycelium cover completely the seed surface.

In our investigation, the main aim was to estimate the occurrence of Alternaria fungi on 16 cultivars of oilseed rape (8 population and 8 hybrid cultivars). Surface disinfected and nondisinfected seeds were incubated on potato dectrose agar (PDA) and on malt extract agar (MA). After 8-9 days of incubation (at 18 ºC and 12 hours photoperiod) the incidence of fungi were observed and identification was performed. The disinfected seeds incubated on both medium were little infected (mean 2.3 % of seeds). Of the obtained isolates, 1.4% were fungi from the genus Alternaria. The most frequently observed was *A. alternata* and *A brassicicola* and to a lesser extent *A brassicae* (mainly on PDA medium). From nondisinfected seeds 16 % on MA and 10% on PDA isolates of fungi were obtained. The incidence of fungi from genus Alternaria on MA medium reached 50% of seeds (6.7% – *A. alternata*, 1.2% – *A brassicicola*), and on PDA over 90% (7% – *A. brassicicola*, 2% – *A. alternata*). On these seeds *A. brassicae* was absent. The occurrence of *Alternaria* spp. on investigated oilseed rape cultivars were similar in the case of seed disinfection and slightly different in case of nondisinfected seeds.