IOBC

International Organisation for Biological Control of Noxious Animals and Plants

Abstract booklet:

Biannual Meeting 2004

IOBC Working Group "Integrated protection in oilseed crops"

at Rothamsted Research, UK

on 30th and 31st of March

Subgroups: Entomology and Phytopathology.

Local organizers:

Prof. Dr. Ingrid Williams,
Dr. Neal Evans
Prof. Dr. Bruce Fitt,
Rothamsted Research, Harpenden, Hertfordshire, UK.
http://www.rothamsted.ac.uk/

Convenor:

Dr. Birger Koopmann,
Institute of Plant Pathology and Plant Protection,
University of Göttingen, Germany.
http://www.uni-goettingen.de/pflanzenpathologie
E-mail: bkoopma@gwdg.de.

Review of progress with the EU-funded project MASTER-MAnagement STrategies for European Rape pests

G-01

I.H. Williams[1], W. Büchs[2], H. Hokkanen[3], Z. Klukowski[4], A. Luik[5], I, Menzler-Hokkanen[3], C. Nilsson[6] & B. Ulber[7]

[1]Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK ingrid.williams@bbsrc.ac.uk; [2]BBA, Institute for Protection in Field Crops & Grassland, Messeweg 11/12, D-38108, Braunschweig, Germany w.buechs@bba.de; [3]University of Helsinki, Department of Applied Biology, Institute of Plant Protection, Box 27, FIN-00014, Finland heikki.hokkanen@helsinki.fi [4]Agric.Univ. Wroclaw, Department of Crop Protection, 50-205 Wroclaw, Cybulskiego str. 32, Poland kluk@ozi.ar.wroc.pl;[5]Estonian Agricultural University, Kreutzwaldi 64, Tartu EE 51017, Estonia luik@eau.ee; [6]Swedish University of Agricultural Sciences, P O Box 44, S-230 53 Alnarp, Sweden christer.nilsson@vv.slu.se; [7]Georg-August University, Institute for Plant Pathology & Crop Protection, Grisebachstr. 6, D-37077 Göttingen, Germany bulber@gwdg.de

Abstract: The EU-funded project 'Integrated pest management strategies incorporating bio-control for European oilseed rape pests' (QLK5-CT-2001-01447) (acronymn MASTER for MAnagement STrategies for European Rape pests) has five objectives: 1. To determine the identity, status and potential of bio-control agents for rape pests in Europe, to increase knowledge of their ecology and identify key factors affecting their efficacy, 2. To develop economically viable, environmentally acceptable IPM strategies for European rape that maximise bio-control of key pests and minimise pesticide use, 3. To determine the socio-economic feasibility, importance and economic efficiency of the IPM strategies in Europe and to assess the socio-economic factors influencing their adoption, 4. To construct a Phenological Model of key pests and their bio-control agents, relating occurrence on the crop to growth stage and climatic/weather conditions, for integration into existing Decision Support systems, 5. To produce Technical Guidelines on the IPM strategies for end-users. The project is targeting the six most important European Rape Pests: *Psylliodes chrysocephala* (cabbage stem flea beetle), *Meligethes aeneus* (pollen beetle), *Ceutorhynchus assimilis* (cabbage seed weevil), *Ceutorhynchus napi* (rape stem weevil), *Ceutorhynchus pallidactylus* (cabbage stem weevil) and *Dasineura brassicae* (brassica pod midge). Information about their key natural enemies has been collated and published. This paper reports on progress during the first two years. Further information can be found on the project website
www.iacr.bbsrc.ac.uk/pie/master/master.htm

SECURE – possibilities for durable resistance to stem canker?

G-02

N. Evans[1], B. Fitt[1], F. van den Bosch[1], M. Eckert[1], Y.-J. Huang[1], S. Pietravalle[1], Z. Karolewski[1,10], T. Rouxel[2]. M.-H. Balesdent[2], S. Ross[2], L. Gout[2], H. Brun[3], D. Andrivon[3], L. Bousset[3], P. Gladders[4], X. Pinochet[5], A. Penaud[5], M. Jedryczka[6], P. Kachlicki[6], A. Stachowiak[6], J. Olechnowicz[6], A. Podlesna[8], I. Happstadius[7] and M. 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: *Leptosphaeria maculans* (anamorph *Phoma lingam*) causes phoma stem canker (blackleg), an oilseed rape (*Brassica napus*) disease of major economic importance in Europe, Australia and Canada. In many European countries, current disease control is heavily reliant on the use of foliar fungicide sprays to control the phoma leaf spotting phase of the epidemic. Although the use of resistant cultivars can offer a viable alternative, the pathogen is highly variable and single-gene resistance tends to break down within a few seasons due to the emergence of new races after the widespread release of resistant cultivars. The SECURE project (StEm Canker of oilseed rape: molecular tools and mathematical modelling to deploy dUrable REsistance; QLK5-CT-2002-01813) aims to deliver a model for deployment of varieties with resistance to *Leptosphaeria maculans* (phoma stem canker/blackleg) to improve durability of resistance and minimise risk that the resistance will break down. The objectives are:

1. To construct a model of the life cycle of *L. maculans* and validate it with existing data.
2. To compare the fitness of virulent/avirulent races of the pathogen and develop genomic analysis of avirulence and virulence loci.
3. To analyse effects of plant genetic background and environmental factors on durability of resistance, in both field and controlled conditions.
4. To model effects of resistance deployment strategies on durability of resistance and recommend a sustainable strategy.

Results will be disseminated and discussed during the course of the project using a website (www.secure.rothamsted.ac.uk), scientific and popular publications and workshops. The SECURE project is supported by the European Commission under the Fifth Framework Programme.

Effects of conservation tillage on harmful organisms and yield of oilseed rape

H. Kreye

Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection in Field Crops and Grassland, Messeweg 11/12, 38108 Braunschweig, Germany

G-03

Abstract: In Germany, conservation tillage systems are becoming more important in agricultural practice. This applies to non-tillage systems only in special cases. Reasons to carry out conservation tillage are e.g. lower labour and machinery costs, protection against erosion, better load-bearing capacity of the soil and the support of soil organisms. In some regions in Germany there is an additional financial support for these tillage systems. Effects on harmful organisms due to soil tillage are known from North America. For this reason a long term field trial was started in 1995 to investigate harmful organisms in a crop rotation of winter oilseed rape-wheat-wheat (barley until 1998). Three types of soil cultivation are tested: ploughing as the standard, conservation tillage by grubbing and no till/direct drilling. Every crop is cultivated every year. We focused on fungal diseases, weeds and slugs.

For *Phoma lingam* or rather the perfect stage *Leptospheria maculans* there was no uniform effect due to the tillage systems. From year to year the highest infection could be found in the ploughing as well as in the direct drilling system.
The effects on sclerotinia stem rot are a little bit different. In 2002, at the beginning of the third rotation we found a level of infection due to tillage. The infection became more severe with decreasing intensity of soil cultivation. It must be shown if this result is repeatable in future growing seasons.
Until now the infection frequency of Verticillium stem disease was very low (<1%) and not influenced by the tillage systems. Other diseases were found only occasionally. They were not influenced by the tillage systems, too.

Effects on the weed population in oilseed rape were found already at the beginning of the trials. Especially the amount of plants of grass weed (*Alopecurus myosuroides*, *Apera spica-venti*, volunteer barley) increased in the systems without ploughing. Sometimes there were fewer grass weeds per m² by direct drilling than by ploughing in the autumn and more spikes of *A. myosiroides* left at harvest. This effect is due to gaps in the oilseed rape population. The amount of other weeds was lowest in the direct drilling system and highest in the trial plots of conservation tillage.

The numbers of slugs increased without ploughing. Especially in the direct drilling trail plots, there was sometimes a severe damage due to slugs. In 2002 there was no oilseed rape left in the late autumn, but that was an extreme case. Slugs are not a problem in every year. In the conservation tillage system the situation was less severe. Tillage at the right time is an effective method to reduce the number of slugs.

The PASSWORD project: a decision support system for managing pests and diseases of winter oilseed rape in the UK

P. Gladders[1], N. Evans[2], B. Fitt[2], K. Walters[3], J. Turner[3], P. Northing[3], K. Sutherland[4]

[1]ADAS Boxworth, Cambridge, CB3 8NN, UK. Email: peter.gladders@adas.co.uk. [2]Rothamsted Research, Harpenden, Herts., AL5 2JQ, U.K. [3]CSL, Sand Hutton,York YO41 1LZ, UK [4]SAC Aberdeen, Aberdeen AB21 9YA, Scotland

G-04

Abstract: Pests and diseases of winter oilseed rape are estimated to cause loses of up to £80 million per annum in the UK. Pesticides are widely used to control them, but treatments are not always used effectively. Decision-making is complex for farmers because of the large seasonal and regional variation in the occurrence of pests and diseases. These issues have been addressed through a project to develop a decision support system known as PASSWORD (Pest And disease control Support System for Winter Oilseed Rape Decisions). This was initiated in October 2000 and continues with a model testing phase up to September 2005.

The PASSWORD system incorporates a decision support system for invertebrate pests of oilseed rape pests (known as DORIS – Decisions on Oilseed Rape Insect Pests) developed by CSL, York, with new disease components for the most important diseases, phoma stem canker (*Leptosphaeria maculans*) and light leaf spot (*Pyrenopeziza brassicae*). Data generated from pest and disease surveys, biological studies and field experiments, particularly since 1986, has been used to develop disease forecasts at the regional level and models for disease development and yield loss at the crop level. During the growing season daily weather data and current crop information is required to run the models and to determine what action is required. The regional forecasts for phoma and light leaf spot are available on the Internet. All the major insect pests of oilseed rape in the UK are adddressed in the pest DSS, including aphids as virus vectors (*Myzus persicae*), cabbage stem flea beetle (*Psylliodes chrysocephala*), rape winter stem weevil (*Ceutorhynchus picitarsis*), pollen beetles (*Meligethes* spp.), cabbage seed weevil (*Ceutorhynchus assimilis*), brassica pod midge (*Dasineura brassicae*) and summer aphid outbreaks (*Brevicoryne brassicae*). The models were developed through close consultation with industry representatives at every stage of DSS construction and validated using a range of techniques.

Comparing fungal diseases on oilseed rape in England, France and Poland

P-01

JS West[1], M Jedryczka[2], A Penaud[3] & BDL Fitt[1]

[1]Rothamsted Research, Harpenden, AL5 2 JQ, UK;
[2] Institute of Plant Genetics, Poznań, Poland;
[3] CETIOM Thiverval-Grignon, France

Abstract: An analysis of data for diseases on untreated oilseed rape plants, predominantly those grown as part of the IMASCORE project in England, France and Poland during the seasons 1997/1998, to 1999/2000, has been used to demonstrate potential changes in disease occurrence due to future climate change. Many climate models predict a slight warming in North-West Europe, with milder winters and warmer summers, but the greatest impact on diseases of oilseed rape is likely to be due to changes in rainfall with increased winter rain but summer droughts. The potential effects of these changes on the timing and severity of various diseases is discussed.

Effect of sulphur, boron, magnesium and fungicide application on occurrence of diseases of spring rape and fungi on seeds

C. Sadowski, A. Łukanowski, L. Lenc

P-02

Abstract: Winter oilseed rape is a main form of rape cultivated in Poland. However, in some years when winter cereals (which are often forecrops of rape) are harvested late because of weather conditions, proper preparation of soil for winter rape is not possible. In such situation the spring form of the crop is cultivated.

During severe, non-snowy winters and also cold early springtime winter rape freezes and the only alternative is the cultivation of spring form of rape. Cropping season of spring rape is relatively short and as a consequence, seed yield is strongly dependent on weather conditions. Threat of fungal infection is also different. Sufficient sulphur content in the soil is also very important for winter rape. This element has an influence on seed and oil yield, nitrogen uptake as well as fatty acid and glucosinolate composition. Sufficient sulphur results in healthy plants. Previous preliminary studies showed a relationship between disease occurrence and sulphur content and also, to a lesser degree, other elements. In light of this, the following experiments were done. The experiments covered different soil and foliar sulphur applications and also treatments which included boron and magnesium foliar applications. Plots which received no sulphur application but did receive fungicide treatment formed control plots.

Disease occurrence was determined each year of the experiements. Downy mildew (*Peronospora parasitca*), powdery mildew (*Erysiphe cruciferarum*) and black spot (*Alternaria* spp.) were noted in higher intensity, while black leg of crucifers (*Phoma lingam*) incidence was lower. Black spot was observed on pods in each experimental year. For this reason, an analysis of *Alternaria* spp. occurrence on seeds was made using the method described by Capelli, Winter and Paul (1998).

In field observations there was no clear effect of sulphur, magnesium and boron application on better health of plants and seed yield. The occurrence of powery mildew, downy mildew and black spot on pods was only limited in one year by fungicide application. Seed analysis showed that the higher intensity of symptoms of black pod spot was correlated with the higher occurrence of *Alternaria brassicae.* Apart from fungicide sprays, other experimental factors had no great impact on the number of seeds infected with *A. brassicae* In eacvh experimental year, a high occurrence of *Alternaria alternata, Cladosporium herbarum*, and *Penicillium* spp. was noted on seeds. Seeds infected with *Alternaria alternata* were often also infected by *Gonatobotrys simplex*.

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

The contribution of cultivar resistance and fungicides to disease control in winter oilseed rape in England

P. Gladders[1], K. Jewell[1] and S. McDonough[2]

[1]ADAS Boxworth, Cambridge, CB3 8NN, UK. Email: peter.gladders@adas.co.uk.
[2]ADAS High Mowthorpe, Malton, North Yorkshire YO17 8BP,UK.

P-03

Abstract: Despite widespread use of fungicides on winter oilseed rape, diseases are estimated to have caused yield losses of up to £80 million/annum in recent years. Fungicide treatments are often ineffective, because they are either not well-timed or unnecessary. Disease incidence and severity show marked seasonal and regional variation, which makes decision making complex and difficult to optimise. Although disease resistant cultivars are available, they have not been fully exploited. A series of eight experiments were done at ADAS Boxworth and ADAS High Mowthorpe during 1999-2002 to investigate the timing of fungicide sprays in relation to cultivar resistance and disease development. ADAS Boxworth is in a high risk area for phoma stem canker (*Leptosphaeria maculans*), whilst ADAS High Mowthorpe has low or moderate incidence of both phoma stem canker and light leaf spot (*Pyrenopeziza brassicae*). Significant yield differences were recorded between cultivars in six experiments, but the highest yields were not associated consistently with high disease resistance. Yield responses to fungicide were significant in five experiments and in each case, two sprays applied in autumn gave comparable yields to a four-spray programme applied in autumn and winter. Fungicides contributed to disease control and yield responses to a greater extent than cultivar resistance. The implications of these results for disease management in the UK will be discussed.

Disease/yield loss analysis for *Sclerotinia* stem rot in winter oilseed rape

S. Dunker, A. von Tiedemann

Institute of Plant Pathology and Plant Protection, Georg-August-University Göttingen, Grisebachstr. 6, 37077 Göttingen, Germany, email atiedem@gwdg.de

P-04

Abstract: *Sclerotinia sclerotiorum* is the most important pathogenic fungus in winter oilseed rape production in Germany. In many growing areas preventive fungicide treatments are carried out to control this disease. To reduce unnecessary fungicide applications, a yield loss related prediction model is to be developed. Within the scope of this project economic damage thresholds of *Sclerotinia sclerotiorum* will be developed.

For the development of the economic damage threshold three different field trials are conducted to investigate the disease/yield loss relations of *Sclerotinia sclerotiorum*. In these field trials different infestation parameters (inoculation date, level of infestation), cropping practices (sowing time, seed density) and the compensation potential are examined on artificially inoculated rapeseed plants. The single plant inoculation is done with the “toothpick-method”, in which wooden toothpicks, previously inoculated with *Sclerotinia sclerotiorum*, are stuck into the leaf axil. Apart from the field trials the economic efficiency of fungicide applications against *Sclerotinia sclerotiorum* is evaluated on the basis of available data from the official field trials of the state extension services since 1991.

The results of the field trials show that the disease/yield loss relationship depends on different factors. The inoculation date has an influence on the yield reaction. Later infections result in lower yield losses. The influence of sowing time and seed density can not yet be closely estimated after two years of field trials. The field trial dealing with the compensation potential of winter oilseed rape shows an increasing thousand seed weight (TSW) with increasing plant reduction. The TSW seems to be a sink for a greater availability of nutrients, water and space due to late plant losses. The economic evaluation of fungicide treatments against *Sclerotinia sclerotiorum* indicates that about 67% of these applications were uneconomic. The mean additional yield of 2,41 dt/ha did not cover the average costs of a fungicide application, which was equivalent to 3,32 dt/ha. The high percentage of uneconomic fungicide treatments against stem rot is due to prophylactic applications.

DNA polymorphism in *Sclerotinia sclerotiorum* isolates from oilseed rape in China

W. Irzykowski [1], J. Sun [2], Q. Li [3], T. Gao [4], S. Hou [3], A. Águedo [5], M. Jedryczka [1]

[1] Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, POLAND, email: wirz@igr.poznan.pl; [2] Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Key Laboratory of Crop Genetics and Breeding, 12 Zhong Guan Cun South Street, Beijing, 100081, CHINA, email: sunjm@mail.caas.net.cn [3] Institute of Crop Research, Anhui Academy of Agricultural Sciences, S40 Nong Ke Rd., Hefei, Anhui, 230031, CHINA; [4] Plant Protection Research Institute, Anhui Academy of Agricultural Sciences, S40 Nong Ke Rd., Hefei, Anhui, 230031, CHINA; [5] Catholic University of Portugal, High School of Biotechnology, Rua Dr. António Bernardino de Almeida 42, 00-072 Porto, PORTUGAL

P-05

Abstract: China is the first producer of oilseed rape in the world (7 mln ha). The crop is grown mostly in provinces along the Yangtse river (mainly Hubei and Anhui). Rapeseed is cultivated in China for its high quality oil for human consumption. It also plays an important role in crop rotation and - due to its profitability - it serves as a good substitute of wheat. The perspectives of the non-agricultural use of this plant make it an even more attractive crop. It is presumed that the acreage of rapeseed will expand in the near future. However, high intensity oilseed rape production is associated with greater pathogen inoculum density, with sclerotinia stem rot (white mould) as the most important problem for cultivation of rapeseed in China. The disease is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary - a filamentous ascomycete with persistent soilborne sclerotia, which serve as the source of primary plant infection. The loss reported in China can reach 30 % of seed yield, and the quality of the remaining harvested seeds is poor. As the pathogen can attack more than 400 agricultural and native plant species, the infested field may convey the disease to subsequent crops.

Although *S. sclerotiorum* is an important pathogen of rapeseed, the isolates originating from infected plants in China and Poland have not been closely studied and characterised so far. The studies concentrated on the pathogenicity of the isolates and the search for new sources of plant tolerance/resistance, with little interest in genetic characterisation of the individual isolates and their populations.

The experiment used 88 isolates of *S. sclerotiorum* from oilseed rape, including 71 isolates from 15 localities in 2 provinces of China (Anhui and Qinghai) and 17 isolates from other countries: Russia (11 isolates), Poland (5), and Austria (1). We used RAPDs to investigate DNA polymorphism amongst these isolates, using three randomly chosen primer sets OPC, OPJ and OPL by Operon Technologies. After screening these primer sets using DNA from five of the isolates, 7 primer pairs showing the highest number of polymorphic bands were chosen for further analysis. The number of unambiguously amplified polymorphic bands ranged from 1 (OPC15) to 18 (OPC02). The frequency of polymorphic bands differed from 1.14 % (polymorphic band present in 1 isolate) to 98.86 % (the same polymorphic band amplified for 87 isolates). 50 polymorphic bands were scored for polymorphism and analysed using TREECON software. Results indicate a close relationship of isolates originating from Hefei (7), whereas other isolates collected at the same sites in other areas of China did not form close clusters. These results coincide with *in vitro* phenotypes of fungal cultures and lower pathogenicity on oilseed rape. The ITS1-5.8s-ITS2 sequences of the isolates from Hefei were identical to the sequences of the other isolates of *S. sclerotiorum* studied.

Field and controlled environment assessment of winter oilseed rape resistance to light leaf spot (*Pyrenopeziza brassicae*)

Z. Karolewski[1,2], D. H. Arkell[1], B. D.L. Fitt[1]

[1]Rothamsted Research, Harpenden, AL5 2JQ, UK; [2]University of Agriculture, Dabrowskiego 159, 60-594 Poznan, Poland

Abstract: *Pyrenopeziza brassicae* is a pathogen that causes severe disease (light leaf spot) worldwide on many cruciferous plants especially oilseed rape (*Brassica napus*). Light leaf spot can cause yield losses of up to 1.5 t/ha per annum, at an estimated cost of more than 30 million Euros in the UK. There is evidence that sources of resistance to light leaf spot in new cultivars are being overwhelmed in a few seasons by changes in the pathogen population. Further evidence for a dynamic *P. brassicae* population comes from changes in UK recommended list resistance ratings for resistance to light leaf spot. The resistance ratings of some widely grown cultivars, for example "Apex" and "Bristol", declined by between 2 and 3 points (on a scale of 9-1) over short periods of between 2 and 5 years. Other cultivars, for example, "Cobra" and "Falcon" showed the opposite trend, with ratings increasing by from 1-2 points over similar periods. The paper describes and compares different methods of screening for resistance to *P. brassicae*, both in the field and in a controlled environment. Results indicate that incubating plants in the lab for 5 days increases infection by between 4% and 15%. Comparisons between other *in situ* and lab screening methods of field-grown oilseed rape plants were less conclusive. Controlled environment screening where 20-day-old oilseed rape seedlings from 28 cultivars were spray inoculated showed no differences in their resistance to *P. brassicae.* It was found that a conidiospore suspension concentration of at least 0.4 x 10^5 ml^{-1} was necessary for the effective inoculation of seedlings in controlled environment.

P-06

Preliminary results on the use of quantitative PCR for assessing resistance to light leaf spot in oilseed rape cultivars

J. Thomas, D. Kenyon and C. Handy

NIAB, Huntingdon Rd., Cambridge, CB03 OLE, UK

Abstract: Resistance to light leaf spot (*Pyrenopeziza brassicae*) on oilseed rape is currently assessed in inoculated field plots for UK National List purposes. The techniques used are time consuming and prone to inaccuracy due to the limitations of visual recording in oilseed rape plots, and the possible cryptic nature of infection in some material. Published primer sequences for *P. brassicae* have been used to quantify the disease in extracts of infected leaves using real-time PCR with a LightCycler™ system. Preliminary results with twelve cultivars indicate some agreement between leaf area infected scores in the winter and amounts of fungal DNA recovered from leaves. However, in some cases the results suggest high levels of fungal development in the absence of visual symptoms. These results will be discussed in relation to the potential for molecular techniques to provide improved methods for resistance evaluation.

P-07

Large-scale survey of race structure of *Leptosphaeria maculans* in France

M.H. Balesdent [(1)], K. Louvard [(1)], X. Pinochet [(2)] and T. Rouxel [(1)].

(1) Institut National de la Recherche Agronomique, PMDV, Route de St-Cyr, 78026 Versailles Cedex, France ;
(2) CETIOM, Centre de Grignon- B.P.4, 78850 Thiverval-Grignon , France

Abstract: Nine avirulence genes were identified recently in *L. maculans*, whose combination theoretically generates up to 512 different races of the fungus. *L. maculans* is a pathogen with a high evolutionary potential to adapt to novel resistance genes as illustrated by the recent breakdown of the resistance gene *Rlm1* in France, where virulent populations of the fungus became strongly established within three growing seasons only. This information illustrated the need for a better knowledge of the fungal populations in terms of race, in order to better use available sources of major gene resistance. The objective of the present study was to get information on *L. maculans* population structure in France, based on a large-scale, appropriate sampling of populations and analysis of the frequency of each of the nine *AvrLm* alleles. Experimental fields, planted with a 'trap' cultivar known to harbour no major resistance gene, were set up in 17 locations. Single-pycnidium isolates were collected from leaf lesions developed in Autumn 2000 and 2001. Only 11 races were identified in the 1787 isolates analysed. One race, virulent on *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4* and *Rlm9* was highly prevalent, representing more than 64% of the populations. Regional disparities were evident at all scale analysed. Some virulent races, such as those harbouring *avrLm5*, were present before the introduction of the corresponding R gene. Consequences for the durable management of resistance will be discussed.

P-08

Frequency of avirulence genes in field populations of *Leptosphaeria maculans* in Germany, UK and Poland

A. Stachowiak [1], J. Olechnowicz [1], M. Jedryczka [1], T. Rouxel [2], M.-H. Balesdent [2], I. Happstadius [3], P. Gladders [4], N. Evans [5]

[1] IGR PAN, Strzeszynska 34, Poznan, 60-479, Poland, mjed@igr.poznan.pl, [2] INRA, Unité PMDV, Route de St Cyr, Versailles 78026, France, [3] Svalöf Weibull AB, Svalöv, SE-268 81, Sweden, [4]ADAS Boxworth, Cambridge, CB3 8NN, UK, [5] Rothamsted Research, Harpenden, Herts., AL5 2JQ, U.K.: neal.evans@bbsrc.ac.uk

Abstract: In September 2002 seven research groups from four European countries (UK, France, Sweden and Poland) began a joint EU-funded project, SECURE (QLK5-CT-2002-01813, see N. Evans *et al*. in this abstract book). The project aims to develop molecular tools and elaborate a mathematical model to deploy durable resistance of oilseed rape to stem canker - one of the most damaging diseases of oilseed rape worldwide. The infection cycle of the disease, caused by the ascomycete *Leptosphaeria maculans* begins when ascospores land on cotyledons and/or leaves of oilseed rape in autumn. This primary inoculum is the product of genetic recombination which results in a high degree of recombination of pathogenicity traits, including the avirulence genes which correspond to resistance genes in the host plant. At the present time, nine resistance genes are known against *L. maculans* in oilseed rape (*Rlm1-Rlm9*). One of the aims of the SECURE project is to study the race structure (= frequency and combination of avirulence alleles) of field populations of *L. maculans* in Europe (600 isolates from six locations).

The isolates characterised in this study originated from field experiments established in the autumn of 2002 at four locations throughout Europe; two in the UK (Boxworth and Rothamsted), one in Poland (Poznań) and one in Germany (Teendorf). The field plots were sown with the spring cultivar Drakkar which was used as a "trapping cultivar" as this cultivar is currently known to lack any resistance genes. Thus, all isolates within the pathogen population would be able to infect plants and develop leaf lesions. Infected leaves with leaf lesions were collected randomly at each location and single-pycnidial isolations were made from the leaf lesions. In total, 274 isolates has been studied (176 from the UK, 77 from Germany and 21 from Poland).

The identification of *L. maculans* races was performed by inoculating each isolate on cotyledons of the extended set of nine differentials which incorporated eight known resistance genes. Assessments were done using the IMASCORE scale 0-6, where a 1-3 result (small to medium size necrotic spots) was interpreted as resistant reaction (i.e. the isolate was avirulent on the cultivar) and a 4-6 score (grey-green tissue collapse without or with the fruiting bodies of the fungus) was recorded as a susceptible plant reaction (i.e; the isolate was virulent). The results of this study showed a similar race structure to that recently described in France (see Balesdent *et al*. in this abstract book), with a lack of isolates possessing the avirulent alleles *AvrLm2, AvrLm3* or *AvrLm9,* and the lack of virulent isolates on *RLm6* or *Rlm7 (avrLm6* or *avrLm7 isolates).* The *AvrLm1* allele ranged from 1.3 % (Germany) to 17.6 % (UK), *AvrLm4* ranged from 1.3 % (Germany) to 6.8 % (UK) and *AvrLm5* ranged from 2.6 % (Germany) to 14.2 % (UK). No *AvrLm1, AvrLm4 or AvrLm5* isolates have been found in the population from Poland so far. The UK population was more variable than the German population, but this may have been because the sample was represented by more isolates collected from two locations, whereas the German population sample consisted of fewer isolates collected at one site.

P-09

Field behaviour of oilseed rape genotypes carrying major resistance genes exposed to different *Leptosphaeria maculans* populations.

H. Brun[1] , V. Huteau[1], M. Ermel[1], F. Eber[2], A. M. Chèvre[2], M. Renard[2]

[1] UMR BiO3P, Domaine de la Motte - BP 35327 – 35653 LE RHEU CEDEX – France ; E-mail : brun@rennes.inra.fr;
[2] UMR APBV, Domaine de la Motte - BP 35327 – 35653 LE RHEU CEDEX - FRANCE

Abstract: Two types of resistance to *Leptosphaeria maculans* exist in oilseed rape (*Brassica napus*) lines : a polygenic quantitative resistance (*e.g.* cv. Darmor) considered as durable and monogenic qualitative resistances conferred by several major resistance genes which have been shown to be specific and non durable. The level of field resistance of a line carrying a major resistance gene (*Rlm*) depends on the presence of the corresponding avirulence gene (*AvrLm*) in the fungus populations. The ratio of avirulence/virulence alleles needed within fungus populations to obtain a good level of resistance is unknown. The objective of the study is to assess the level of field resistance obtained from different *Rlm* genes depending on the relative frequency of the corresponding *AvrLm* genes within the fungus populations tested.
Six different major resistance genes were tested using the lines 'EurolMX' (*Rlm2*, *Rlm3*, *Rlm6*), 'Falcon' (*Rlm4*), 'Capitol' (*Rlm1, Rlm3*), 'SMX' (*Rlm1*, *Rlm3*, *Rlm6*), 'Eurol' (*Rlm2*, *Rlm3*), 'Samouraï' (*Rlm2*) and 'Darmor' (*Rlm9*). These lines were grown in three field trials distant of at least 1 km, contaminated each by one population previously obtained by field recurrent selection of *L. maculans* isolates on plant residue of : either (i) susceptible oilseed rape lines (without efficient *Rlm* genes), or (ii) 'EurolMX' line, or (iii) 'SMX' line. Alleles frequencies in each of these populations were assessed on a sample of isolates characterised under controlled conditions on a differential host set carrying the major genes leading to detect the presence of *AvrLm1, 2, 3, 4, 6* and *9* in the isolates. The level of disease under field conditions was assessed for each line by scoring leaf lesions in the autumn and stem base canker in June.
Within the three fungus populations no *AvLm3, AvrLm9* and a very few *AvLm2* isolates were detected. Exposed to each of these fungus populations under field conditions 'Eurol' was always susceptible and 'Darmor' resistant to stem base canker but susceptible to leaf lesions. Within the first population, 20.8%, 25% and 87.5% of isolates carried *AvrLm1, AvrLm4* and *AvrLm6*, respectively. Facing this population 'EurolMX' was highly resistant, 'Capitol' and 'Falcon' highly susceptible to both leaf lesions and stem canker. Within the second population, 83.7% of *AvrLm1*, 2.3% *Avrlm2*, 11.6% *AvrLm4*, no *AvrLm6* isolates were detected. Exposed to this population, 'EurolMX', 'Capitol' and 'Falcon' were susceptible to stem base canker even though 'Capitol' had less leaf lesions than other lines in autumn. Within the third population no *AvrLm1* and *AvrLm6* isolates were detected and 63.1% of isolates carried *AvrLm4.* 'Samouraï' and 'SMX' were susceptible but 'Falcon' was resistant to stem base canker. From the data, no clear relationship was detected between the level of resistance observed in the field conferred by a specific *Rlm* gene and the relative frequency of the corresponding *AvrLm* gene within fungus populations. It seems to depend on the *Rlm* gene considered.
This study is a first attempt to connect the relative frequency of avirulence/virulence alleles within *L. maculans* populations to a level of field resistance efficiency of several major resistance genes.

P-10

Identification of specific resistance to Phoma among oilseed rape varieties: interest for variety testing and for the promotion of a first step of a durable management of resistances

X. Pinochet, M.-H. Balesdent, F.Salvi, E. Mestries and T. Rouxel

CETIOM, DS Innovations, BP4, 78850 Thiverval-Grignon, INRA, Unité PMDV, Route de St Cyr, 78000 Versailles

Abstract: Blackleg (*Leptosphaeria maculans*) is the main disease of oilseed rape to date. Breeding resistant cultivars is still the major way used for its control. The recent break down of a specific resistance widely used during the nineties, the available knowledge, and the fungal material available support the distinction between quantitative and specific resistances during genotypes evaluations. This paper presents recent results of specific resistance identification in commercial varieties and suggests an association with more classical tests to promote a first step of durable management of plant resistances based on alternation and diversification of cultivars in time and in space.

P-11

Feasibility of using quantitative PCR for assessing resistance to stem canker in oilseed rape cultivars

D. Kenyon, J. Thomas, C. Handy

NIAB, Huntingdon Rd., Cambridge, CB03 OLE, UK

Abstract: Stem canker (*Leptosphaeria maculans*) is the most damaging disease of oilseed rape in the major growing areas of the UK. Disease control through the exploitation of inherent resistance factors is now increasingly required in order to realise both the economic and environmental benefits desired by government, the industry and the public. Information on resistance is generated in “Value for Cultivation and Use” trials using an established technique in which stems are destructively sampled and the degree of symptom development assessed visually before harvest. The disease cycle of stem canker involves a long period of symptomless systemic growth in the autumn and winter, and the rate of systemic growth is likely to be highly correlated with the final expression of symptom severity. A molecular diagnostic method of measuring systemic growth would thus provide a highly specific and sensitive means of measuring cultivar resistance. This paper describes the development of a method for the detection of systemic growth of *L. maculans* in oilseed rape using quantitative real-time PCR, and its potential to replace the current field based assessments.

A modelling approach to durability of resistance of winter oilseed rape to stem canker

S. Pietravalle[*], F. van den Bosch [*] and N. Evans[+];

[*]Biomathematics Unit and [+]Plant Pathogen Interactions Division, Rothamsted Research, Harpenden AL5 2JQ, UK

Abstract: *Leptosphaeria maculans*, the cause of stem canker, is the most serious disease of oilseed rape worldwide. Although the use of fungicides remains the main means of control of the disease, the use of resistant cultivars provides an environmentally friendly alternative. However, when a resistant cultivar is introduced into the field, it is only a matter of time before resistance is broken, but the time the breakdown process takes may vary considerably.

In this work, a modelling approach of durability of resistance was developed using ordinary differential equations with transition rules. Starting from a generic model with two strains of the pathogen (virulent and avirulent) and two cultivars (resistant and susceptible), the study focuses on the case of monocyclic diseases. However, the approach taken was not to derive a complete predictive model in order to validate the model against data, instead we investigated the effects of a large range of epidemiological and environmental factors on durability of resistance. In this way we hope to develop optimal strategies for the deployment of resistant cultivars in the field.

Three measures of durability will be discussed. Firstly, “time until take-over” is defined as the number of seasons needed for the virulent pathogen to reach a given threshold/frequency in the population after its first appearance. Secondly, the “estimated yield gain” is defined as the total number of days when the crop is not infected gained through the use of the resistant cultivar. Lastly, “time of usefulness” is defined as the number of seasons while the estimated gain in yield per plant due to the use of the resistant crop is significant. Finally, a way to introduce partial resistance into the model will be discussed.

LeptoNet and SPEC - new projects supporting the control of stem canker of oilseed rape in Poland

M. Jędryczka [1], R. Matysiak [2], K. Graham [2]

[1] Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, POLAND; e-mail: mjed@igr.poznan.pl [2] DuPont-Poland, Powazkowska 44c, 01-797 Warszawa, POLAND

Abstract: Stem canker of oilseed rape in Poland has been observed since the begining of 1980s. The first outbreak of the disease was observed in north-west Poland. However, the disease is now pandemic across the whole of Poland. For several years the phoma leaf spot symptoms on leaves were not observed frequently. Usually the first visible symptoms appeared on stems in early to mid-June at the green pod growth stage. These early symptoms developed very fast and sometimes the plants were killed before before harvest. A survey of the population of *Leptosphaeria maculans* in Poland showed that most of fungal strains isolated from infected tissues belonged to the less aggressive type of the pathogen (B-type or Siro^0, now identified as *Leptosphaeria biglobosa*). Between 1984 and 1996, only 5 % of the isolates represented the highly aggressive type of *L. maculans* (A-type or Siro+). However, since the mid-1990s, symptoms on leaves and plants at the flowering stage were observed more and more frequently and this coincided with an increased number of isolations of the aggressive type *L. maculans*.

Two new initiatives have been recently undertaken to support the control of stem canker in Poland. The first one, commenced in the autumn 2003 is a network of fields with "trapping" cultivars with no resistance genes to *L. maculans.* The idea is based on the system introduced by INRA in France (2000), followed by the experiments performed for the EU FP5 project SECURE (QLK5-CT-2002-01813). At present the Polish network comprises fields with spring cv. Drakkar and winter cv. Darmor, grown in 13 places in different regions. The system is supported by the Central Cultivar Testing Station (5 fields), the Institute of Plant Genetics PAS (2 fields), the Institute of Soil Science and Plant Cultivation (2 fields), the Agricultural University of Poznan (1 field), the Institute of Plant Protection (1 field) and two oilseed rape breeding stations (1 field each). Leaves and stems of infected oilseed rape plants will be collected and studied for the composition of pathogenicity groups/races of *L. maculans* and their distribution among the regions of Poland.

The SPEC system, whose name originates from the Polish abbreviation of the System of Forecasting of Disease Epidemics (*pol*. System Prognozowania Epidemii Chorob) will be initiated in September 2004. It will comprise five Burkard traps located in the main oilseed rape growing regions of Poland. Four traps will be provided by DuPont and one by the IPG PAS. The network of traps will allow the study of the release of *L. maculans* ascospores in different regions of Poland. The coordination of both systems, the collection and isolation of fungal cultures, the recognition of pathogenicity groups and races of the pathogen and the studies of the maturation of *L. maculans* in Poland will be performed at the IPG PAS with financial support from DuPont.

The results will be available for farmers and all representatives of the plant protection services in Poland, through a non-profit web site and through the use of email and SMS text messages. More than 2000 farmers and representatives of agricultural services were informed about the benefits of Lepto-net and SPEC, at conferences organised by DuPont and IPG (project PAGEN) in the autumn of 2003 and winter 2004. The addresses of all parties interested in the results are recorded in a database which makes it possible for us to send the requested information to the end-users of our research.

P-14

Development of an 'ascospore shower' method for inoculating oilseed rape leaves with *Leptosphaeria maculans*

Y.-J. Huang [a], M.-H. Balesdent [b], N. Evans [a], B. Fitt [a]

[a] *Rothamsted Research, Harpenden, Herts AL5 2JQ, UK*
[b] *INRA-Pathologie Végétale, Route de St Cyr, F-78026 Versailles, Cedex, France*

Abstract: Two types of inoculum (ascospore and conidia) and four inoculation methods (spore suspension droplet on unwounded leaf, spore suspension droplet on wounded sites of leaf, spore suspension sprayed onto leaves and dry ascospore 'raining down' onto leaves) were used to develop an efficient method for inoculation with *L. maculans* when inoculum is limited. Ascospore was more infective than conidia. All the plants inoculated with ascospores, whether the ascospores were from natural conditions or from defined crosses between JN2/JN3, produced phoma leaf spots regardless the inoculation methods. Plants inoculated with conidial suspensions by droplet inoculation on wounded sites and by spraying produced lesions, while plants inoculated by droplet inoculation without wounding the leaf produced no lesions. For droplet inoculation, the infection efficiency of ascospores was 100% while the infection efficiency of conidia was 56.1%. For spray inoculation, nine ascospores were needed to cause one lesion whereas 2.4×10^6 conidia were needed to cause one lesion. For inoculation with ascospores from crosses between JN2/JN3, 10 pseudothecia were needed to make 200 µL ascospore suspension at the required concentration, whereas three pseudothecia produced 3.5 lesions per plant when they were used to inoculate three plants by the ascospore 'shower' method. Since it is difficult to get enough ascospores from crosses to make ascospore suspension, 'ascospore shower' was chosen to study the fitness of near isogenic isolates of *L. maculans*.

Spatial aspects of *Leptosphaeria maculans* (phoma stem canker) epidemiology

P-16

N. Evans [*], A. Baierl [+], J.S. Steed [*], Y.-J. Huang [*] and B.D.L. Fitt [*]

[*]Plant Pathogen Interactions Division, and [+]Biomathematics Unit, Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK E-mail: neal.evans@bbsrc.ac.uk

Abstract: *Leptosphaeria maculans* (anamorph *Phoma lingam*), the causal agent of phoma stem canker (blackleg) is a serious disease of oilseed rape (*Brassica napus*). Epidemics are initiated in late autumn when the pathogen infects the leaves of the young crop. The pathogen grows systemically down the petiole to cause infections in the stem by late winter. Stem infections develop during the spring period to produce cankers which reduce yield before harvest. It is thought that initial infections are initiated by wind-dispersed ascospores and that secondary rain-splashed pycnidiospores do not play a role in the epidemiology of the disease within the UK.

Data were collected on 18 December 2002 and 15 January 2003 using a "grid" sampling method to investigate the spatial distribution of phoma leaf spotting during the winter period of the growing season. These data indicated that distributions of % leaves with phoma leaf spotting fitted a binomial distribution and did not indicate any local aggregation of disease. To investigate auto-correlation between disease measurements and distance between samples, variograms were estimated with the data for both assessments. For the January assessment, the variogram did not indicate any spatial auto-correlation. The variogram for the December assessment showed a continuous increase in variance of disease measurements up to an inter-sampling distance of about 15 m. This suggested that more data on a finer grid are needed to make more general statements about spatial distribution of phoma leaf spotting.

A dataset, collected by a "cluster" sampling technique using "microplots" throughout the 1998/99 and 1999/2000 seasons was analysed to investigate the spatial dynamics of the epidemic in more detail. Data at several sampling dates indicated that the incidence of plants affected was significantly different from a binomial distribution, indicating over-dispersal suggesting local aggregation of infection between near-neighbour plants. However, there were differences between similar sampling dates in the two seasons of the study and the pattern was not consistent, presumably because of leaf fall. The results suggest that further research is required before the spatial dynamics of epidemic development and the specific role of ascospores and pycnidiospores of *L. maculans* during the epidemic can be fully explained.

Effects of temperature and wetness on *L. maculans* symptom development on cotyledons of oilseed rape with different resistance genes

P-17

J. Olechnowicz[1] , A. Stachowiak[1] , M. Jędryczka[1], A.-M. Chèvre[2], M. Renard[2]

[1] Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, POLAND e-mail: mjed@igr.poznan.pl; [2] UMR APBV, Domaine de la Motte BP 35327, 35653 Le Rheu Cedex, FRANCE

Abstract: Infections of winter oilseed rape plants by *Leptosphaeria maculans* are initiated by ascospores of the pathogen in the autumn. Ascospores are released from mature pseudothecia - fruiting bodies of the sexual stage, that form on contaminated stubble during the previous season. The rate of pseudothecia maturation depends on weather conditions, in particular on rainfall and humidity of the soil on which the stubble is deposited. In wet summers, pseudothecia mature faster and they are ready to release ascospores at the beginning of autumn. For example, in the autumn of 2003 in the region of Wielkopolska, central-west Poland, the first ascospores trapped by a Burkard trap were found on 9-12 September. At this time most plants of winter oilseed rape were at the cotyledon stage. The experiments presented in this study were designed to show the effects of temperature and wetness on the development of disease symptoms on plants infected with ascospores of the pathogen, and the effect of resistance genes expressed in some new winter oilseed rape lines.

Artificial inoculation was performed on two cultivars and two lines of winter oilseed rape, bearing different specific *Rlm* resistance genes. The cultivar Darmor has one known resistance gene *RLm9* and a polygenic resistance efficient at the adult stage; the cv. Eurol has two known resistance genes *RLm2 and RLm3*. Two lines named DarmorMX and EurolMX carry the resistance gene *RLm6* introgressed into cvs. Darmor and Eurol, from *Brassica juncea*. The inoculation was performed in controlled environment cabinets. The inoculation of plants was done 12 days after sowing at 15 ^{0}C and 20 ^{0}C using two methods: 1) a suspension of ascospores of *L. maculans* at a concentration of 1 x 10 ^{6} spores/mL; 2) an “ascospore shower” directly from fragments of winter oilseed rape stubble bearing pseudothecia of *L. maculans*. The fruiting bodies of the fungus present on the stubble originated from natural infection (harvested in 2003 at Rothamsted, UK). For both types of inoculation there were three different lengths of wetness duration after inoculation: 12 h, 24 h and 48 h of full coverage of plants with plastic hoods. For inoculation with the ascospore suspension, two other factors were introduced: 1) intact cotyledons, 2) cotyledons wounded with a needle before the application of a 10 μL droplet of the ascospore suspension.

The results clearly demonstrated the efficiency of the introduced resistance gene *RLm6*. In all variants of the experiment, the cultivars Eurol and Darmor were highly infected, whereas the cultivars with the additional gene *RLm6* showed little necrotic symptoms. In case of the presence of *RLm6* the average size of the biggest symptom was 4,9 mm necrotic spot, even in conditions conducive to disease development (20 ^{0}C, 48 h wetness after inoculation, 14 days post inoculation, wounded cotyledon of cv. DarmorMX). The symptoms for cv. Darmor were in all cases slightly more severe than for cv. Eurol (e.g. 14,85 mm mean symptom diameter for Eurol and 16,37 mm for Darmor, 14 dpi, 20 ^{0}C, 48 h wetness). Symptom development was faster and more severe with increasing temperatures and lengths of wetness duration post inoculation. Differences in severity of symptom development between wounded and unwounded cotyledons reduced with increasing wetness duration post inoculation. However, symptom severity was always greater on wounded cotyledons than on intact cotyledons inoculated with *L. maculans* ascospores .

Leptosphaeria maculans, *L. biglobosa* and fungicides: preliminary results from in vitro and field observations

M. Eckert, B.D.L. Fitt, A. Selley

[1]*Rothamsted Research, PPI Division, Harpenden, Herts., AL5 4SE, UK*
[2]*DuPont, Wedgwood Way, Stevenage, Herts., SG1 4QN, UK*
e-mail: maria.eckert@bbsrc.ac.uk

P-18

Abstract: Since 1995, phoma stem canker has been the dominant disease of oilseed rape in the UK, resulting in estimated losses of more than £30M each year. The disease is caused by a fungal species complex, comprising at least two species (*Leptosphaeria maculans* and *L. biglobosa*). In the UK, disease control relies on the use of triazole fungicides, and over 90% of winter oilseed rape crops receive one or more fungicide applications within a growing season. Despite the frequent use of fungicides, little is known about the effects these chemicals have on disease dynamics and pathogen population structure. We aimed to investigate such effects and report first results.

The susceptibility of *L. maculans* and *L. biglobosa* to fungicides commonly used in the UK was tested *in vitro*. Isolates were tested on medium containing flusilazole, tebuconazole or difenconazole. The radius of mycelial growth was recorded and effective dose at which 50% of growth was inhibited (ED_{50}) determined. There was a significant difference in susceptibility to triazole fungicides between *L. maculans* and *L. biglobosa*. The effects of fungicide application on the pathogen population structure were investigated in a field experiment at Rothamsted Research in 2002/2003. Winter oilseed rape cv. Apex was grown in randomised block experiments with three replicates of untreated, early fungicide treated or late fungicide treated plots. Flusilazole + carbendazim was applied as Punch C when 10% of plants showed phoma leaf spots (early treatment) or 10 weeks later (late treatment). Shortly before harvest, 60 diseased plants were sampled from each treatment, assessed for stem canker and tissue samples taken from the stem base and upper stems. Disease due to *L. maculans* or *L. biglobosa* was assessed by cultural isolation of the pathogen and by extraction of DNA from diseased tissue followed by PCR analysis. Results indicate a significant effect of the early fungicide treatment on the pathogen population, where a reduced number of *L. biglobosa* isolates was detected on basal and upper stems. Stem canker severity was reduced more by the late treatment.

Molecular characterization of Portuguese isolates of *Leptosphaeria maculans* using PCR-ISSR and RAPD markers

J.S. Godinho[1]; M. H.Balesdent[2]; E. Mendes-Pereira.[2] & J.S. Dias[1]

[1]*Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal.*

[2] *Unité Phytopathologie et Méthodologies de la Détection Versailles (PMDV), Institut National de la Recherche Agronomique, Route de Saint Cyr, F-78026 Versailles cedex, France.*

P-19

Abstract: PCR-ISSR and RAPD markers were used to study the genetic relationships of 30 isolates of *Leptospheria maculans* including 18 Portuguese isolates from Beja region (south Portugal). Foreign isolates were also included to determine the relationships of Portuguese isolates to other related isolates. Cluster and principal components analyses were conducted using a package of computer programs and PCR-ISSR and RAPD data from amplification with selected ISSR primers and RAPD markers, detecting 234 polymorphic fragments. The results showed that the 30 isolates clustered into two distinct groups (Tox+ and Tox^0 isolates) and 4 subgroups: i) a large and compact subgroup containing all the Tox+ *L. maculans* "brassicaceae" isolates including all the Portuguese isolates; ii) the unique "Lepidium" Tox+ isolate; iii) a relatively heterogeneous subgroup with the Tox^0 NA2 *L. biglobosa* "canadensis" isolates; and iv) a dispersed subgroup with the other Tox^0 NA1 and NA3 *L. bigolosa* isolates and NA2 *L. bigolosa* "erysimii" isolate. There is low similarity between these three isolates. The Portuguese and foreign Tox+ *L. maculans* "brassicaceae" isolates could be further divided into phenetic groupings/clusters. These groupings of Portuguese isolates based on PCR-ISSR and RAPD data do not corresponded to their pathogenicity groups revelead by plant differentials.

Thlaspi arvense, a source of A-type isolates of *Phoma lingam*?

M.R. Islam[1], R.K. Gugel[2], G. Séguin-Swartz[2] and B. Koopmann[1]

[1]*Institute of Plant Pathology and Plant Protection, Georg-August University of Göttingen, Grisebachstr. 6, 37077 Göttingen, Germany.*
[2]*Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, Saskatchewan, S7N 0X2, Canada*

P-20

Abstract: Blackleg or stem canker, caused by the fungus *Phoma lingam*, is a serious disease of oilseed rape (*Brassica napus*) around the world. Numerous strains have been isolated from oilseed rape (OSR) and characterized. In contrast, only limited information is available regarding isolates from cruciferous weeds like stinkweed (*Thlaspi arvense*), hedge mustard (*Sisymbrium officinale*), *Lepidium* spp., *Erysimum* spp. and others that occur in oilseed producing regions. Most isolates described so far resemble B-type isolates classified to be non-aggressive on *B. napus*; B-type isolates do not produce host nonspecific toxins called sirodesmins (SIRO°, Tox°), which belong to the chemical family of dioxo-piperazines. In contrast, A-type isolates are classified to be aggressive on oilseed rape, producing sirodesmins ($SIRO^+$, TOX^+). A-type isolates are associated with stem canker and are believed to have a major impact on yield of OSR compared to B-type isolates. Cruciferous weeds may serve as a green bridge for A-type isolates and may, therefore, be important with respect to epidemiology of *Phoma lingam*. Therefore, the objective of this study was to investigate 18 isolates obtained from seeds of Canadian accessions of *Thlapsi arvense.* These isolates were characterized by studying phenotypic and genotypic traits and compared with nine reference isolates from the "*International Blackleg of Crucifers Network*" (IBCN) displaying the known variability of *Phoma lingam*.
Radial growth was investigated on both V8- and malt extract agar (V8/MEA). Growth rates of the Thlaspi isolates displayed a continuum (mean values: 0.71/0.45 cm/d) between both A-type (0.53/0.23 cm/d) and B-type NA1 (0.75/0.46) reference strains. Some Thlaspi isolates also grew slightly faster on MEA than the B-type NA1 isolate. All Thlaspi isolates produced pigments of different color in Czapek-Dox broth amended with yeast extract. For the Thlaspi isolates tested, no *in-vitro* production of sirodesmins was detected using thin layer chromatography. On the basis of these phenotypic characters, Thlaspi isolates are related to B-group isolates. VNTR-, ERIC-PCR and ITS-analyses revealed two different Thlaspi groups, which are distinct from IBCN A-group, B-group (NA1, NA2, NA3, Thlaspi, Sisymbrium, Erysimum, Australia) and Lepidium reference isolates. Finally, aggressiveness tests on OSR cv. Lirabon were performed. None of the Thlaspi isolates was virulent on cotyledons of the susceptible differential Lirabon. Aggressiveness of Thlaspi and reference isolates also were compared on stems of this cultivar. Stems were inoculated in the greenhouse with mycelium plugs. Disease severity was assessed 49 dpi using a modified rating scale described by Kutcher et al. (1993). All Thlaspi isolates were significantly less aggressive than the A-type reference. In conclusion, there is so far no indication that *Thlaspi arvense* serves as a reservoir for A-type isolates.

Sirodesmins in tissues of infected rape plants revisited

P. Kachlicki

Institute of Plant Genetics, Polish Academy of Sciences, Strzeszynska 34, 60-479 Poznan, Poland

Abstract: Sirodesmin PL and other structurally related compounds are well known phytotoxins produced *in vitro* by fungus *Leptosphaeria maculans* (Desm.) Ces. et de Not (vegetative stage: *Phoma lingam* (Tode ex Fr.) Desm.). These phytotoxins are not host-specific and their application on leaves of plants from various species causes formation of necrotic spots. However, there still is some doubt regarding the role of these compounds during the process of infection of *Brassica napus* plants. Sock et al. (1995) have found sirodesmin PL to be present in leaves of *B. napus* infected with *L. maculans*, but in these experiments plants after the inoculation had to be placed in darkness for 4 days. On the other hand, according to the studies of MSC Pedras and her team (Pedras and Biesenthal, 1998), sirodesmin PL is regarded to be not present in tissues of the infected rape plants.

This study has been performed using the HPLC instrument connected to mass spectrometer (Esquire 3000, Bruker, Germany). The high sensitivity of MS enables the detection and identification of substances present in amounts as low as 1×10^{-12} g. The plant material used were cotyledons of oilseed rape seedlings used in the Williams's test from cultivars differing in their resistance to *L. maculans* (Westar, Columbus, Darmor MX) and leaves of naturally infected plants as well as the non-infected controls. Secondary metabolites were extracted from the freeze-dried plant tissue with methanol-water solution 70% and purified using solid phase extraction columns C_{18}. The HPLC chromatography was performed in the reversed phase system using water-acetonitrile gradient elution and compounds were identified according to their ESI MS and MS/MS (up to MS^4) spectra.

The experiments demonstrated the presence of sirodesmins PL and J in cotyledons exhibiting the susceptible reaction (Westar, Columbus) but was neither found in those resistant to the infection (Darmor MX) nor in the not infected seedlings. Similarly, rape leaves with symptoms of *L. maculans* infection contained both sirodesmins which were not found in the healthy leaves.

P-21

Pedras MSC, Biesenthal CJ (1998). Production of the host-selective phytotoxin phomalide by isolates of *Leptosphaeria maculans* and its correlation with sirodesmin PL production. Can. J. Microbiol. 44: 547-553.

Sock J., Mennen H., Hoppe HH. (1995). Pathogenicity of sirodesmin-deficient mutants of *Leptosphaeria maculans*. *Proc. 9th Int. Rapeseed Congress, Cambridge, UK*: 613-615.

"*Agrobacterium tumefaciens*-mediated transformation of *Leptosphaeria maculans*"

M. Meyer, F. Blaise, E. Remy, L. Zhou, C. Tourneur, T. Rouxel and M.-H. Balesdent

Institut National de la Recherche Agronomique, PMDV and Biologie cellulaire (fith author), Route de St-Cyr, 78026 Versailles Cedex, France

Leptosphaeria maculans (anamorph: *Phoma lingam)*, a severe phytopathogen of oilseed *Brassica* spp causing stem canker, was transformed successfully using *Agrobacterium*-mediated transformation. The selection marker employed was the *hph* gene conferring resistance to hygromycin B. Transformation with *hph* and green fluorescent protein (GFP) genes was also performed. Transformation frequency of up to 120 transformants per 10^7 conidia were obtained. The presence of the *hph* gene was checked by PCR and Southern analysis. The majority of *L. maculans* transformants contained a single-copy integration of the marker gene at different chromosomal sites. Transformants were mitotically and meiotically stable. GFP expression was observed in-vivo with the GFP-tagged strain. Agrobacterium-mediated transformation is now routinely used to generate a large collection of mutants that will allow us to identify genes which are paramount for *Leptosphaeria maculans* pathogenicity to oilseed rape.

P-22

Screening of *B. napus* with *Xanthomonas campestris* pv. *campestris* and *Leptosphaeria maculans*

J.S. Dias, B. Ribeiro & J. Godinho

Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

Abstract: Ninety accessions of *Brassica napus* were screened against two isolates of *Xanthomonas campestris* pv. c*ampestris*, (Xcc524, race 4 and Xcc512, race 1), using inoculation by injection of the 10^8 cfu/ml bacterial suspension directly into the leaf's veins of 3-4 true leaves plants. All the ninety accessions were found to be very susceptible to isolate Xcc512. Twenty-three accessions presented more than 25% of the plants resistant to isolate Xcc524. The most resistant accessions were CGN14113, ISA156, ISA78, BRS0054 and ISA666. These accessions were further screened against other nine Portuguese and foreign isolates (Xcc513, race 0; Xcc545=PHW117, race 1; Xcc459=2D520, race 2; and Xcc510, Xcc516, Xcc501, Xcc530, Xcc547=HRI1279a, and Xcc550=Dickson171 all race 4). All the accessions, except CGN14113 that presented moderate levels of resistance to Xcc516, were susceptible to isolates Xcc513, Xcc545, Xcc459, Xcc510 and Xcc516. GGN14113 was also the only accession presenting resistance to isolates Xcc501, Xcc530, Xcc547 and Xcc550.

The twenty-three resistant accessions to *Xcc* were further screened at the cotyledons against four isolates of *Leptosphaeria maculans* (BBA62908, PG2-A3; T12aD34, PG4-A1; PT01, PG4-A1; and V11.1.1, PG4-A1). Only two accessions ISA666 and NU51661/2 were resistant to the four isolates. Accessions ISA302 and CGN14113, ISA156, ISA78, BRS0054, ISA381, and ISA305 presented respectively resistance and moderate resistance to isolate BBA62908. Accession BRS0085 was resistant to isolate V11.1.1 and moderately resistant to the other isolates and accessions ISA 78 and BRS0061 were moderately resistant to isolates T12aD34 and PT01 and V11.1.1, respectively. All the other accessions were susceptible to all the isolates.

ISA666 and NU51661/2, respectively with 54% and 42% of resistant plants to isolate Xcc524 and resistant to all the four isolates of *L. maculans* studied, are new interesting sources of resistance for *B. napus* breeding.

P-23

The exploitation of genetic resources of *Brassica juncea* for resistance to *Xanthomonas campestris* pv. *campestris*

J. S. Dias & J.P. Paiva

Instituto Superior de Agronomia, Technical University of Lisbon, Tapada da Ajuda, 1349-017 Lisboa, Portugal.

Abstract: Fifty accessions of *Brassica juncea* were screened against four isolates of *Xanthomonas campestris* pv. c*ampestris*, Xcc524 (race 4), Xcc512 (race 1), Xcc459 (=2D520, race 2) and Xcc521 (race 0), using inoculation by injection of the 10^8 cfu/ml bacterial suspension directly into the leaf's veins of 3-4 true leaves plants. It was found that 92%, 90%, 16% and 0% of the accessions were respectively resistant to those isolates. The most resistant accessions were the lines "HRI7677/1" and "HRI7677/3", and the cultivars "Leaf Mustard", "West Lake", "Late Head" that were resistant to all those isolates, except the race 0 Xcc521 isolate.
Twelve selected mustards were screened at the 3-4 thrue leaves, using the same technique, with fourteen isolates of *X. campestris* pv. *campestris*, including isolates races 0, 1, 2 and 4 isolated from *B. oleracea* and *B. rapa*. In general the 12 accessions were resistant to isolates races 1 and 4 and susceptible to isolates races 0 and 2. The isolates isolated from *B. rapa* were the most virulent in the accessions tested. Only accessions "HRI7677/1" and "HRI7677/3" were high resistant to two isolates race 0 (Xcc513 and Xcc523) and to isolate race 2 Xcc549 which is new since the few mustards screened until now in previous studies were only considered resistants to isolates races 1, 3 and 4. This study confirms the high potential of mustards as sources of resistance of black rot and has identified two new accessions with a high valuable resistance.

P-24

Effects of a turnip rape trap crop on the spatial distribution of *Meligethes aeneus* in oilseed rape

E-01

S. M. Cook[1], N. P. Watts[1], F. Hunter[1], L. E. Smart[2] and I. H. Williams[1]

[1]Plant & Invertebrate Ecology Division, [2]Biological Chemistry Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ. UK.

Abstract: Alternative control strategies for insect pests of oilseed rape (*Brassica napus*) are needed to help reduce the current reliance on insecticide treatments. We investigated the effect of a turnip rape (*Brassica rapa*) trap crop border on the abundance and spatial distribution of a major oilseed rape pest, the pollen beetle (*Meligethes aeneus*) in oilseed rape plots. Pollen beetle abundance was recorded throughout the growing season at spatially referenced sampling points evenly arranged within the plots. Growth stage of the plants in the plot centres and borders were also assessed. The turnip rape developed more quickly than the oilseed rape. Pollen beetle abundance was greater overall in the plot with a turnip rape border than in the control plot. However, beetles were retained in the turnip rape border and consequently the population in the oilseed rape plot centre was lower than in the plot without a turnip rape trap crop. This effect lasted throughout the pollen beetle damage-susceptible stage of oilseed rape (green-yellow bud) and until the oilseed rape was in late flower and the turnip rape trap crop was in pod. The usefulness of this trap-cropping strategy for control of the pollen beetle is discussed.

Spatial dynamics of pollen beetles (*Meligethes aeneus*) in relation to inflorescence growth stage within a simulated trap crop system for oilseed rape

D. Frearson, A.W. Ferguson, J. Campbell and I.H. Williams

E-02

Abstract: Pollen beetles within oilseed rape are conventionally controlled using pesticides, but their use could be reduced by using trap crops to concentrate the pests. One potential strategy uses a trap crop of attractive early-flowering plants to protect the more susceptible late-bud stage of the main crop. Here we use semi-field arrays of potted oilseed rape plants to investigate this strategy, observing the development of distributions of pollen beetles in a simulated trap crop system. In two experiments, total pollen beetle populations were counted on every plant, and total racemes in bud and racemes in flower were counted to assess host plant resources and cues. Inflorescence growth stage characteristics were shown to be important in determining the spatial distributions of pollen beetles. The beetles were usually spatially associated with plants with more racemes in bud and/or in flower. The trap crop maintained a significant edge distribution of pollen beetles for 7-10 days. While flowering racemes provided strong cues for immigrating pollen beetles, the abundance of buds was a more important determinant of residence time on the plants. This highlights the importance of establishing trap crops that are both attractive and provide resources that retain the pest.

Trap plants to avoid insecticide application against pollen beetles in oilseed rape

C. Nilsson

Department of Plant Science, Swedish University of Agricultural Sciences, Box 44, SE-230 53 Alnarp

E-03

Abstract. Rape seed was mixed with 2% turnip rape seed to produce trap plants within the oilseed rape crop. The turnip rape plants were up to 10 days faster in there development, which made theme much more attractive to pollen beetles at early growth stages of the rape crop. At the most vulnerable growth stage of the rape plants, the turnip rape plants attracted 10 times more beetles. As the threshold for control usually not is exceeded with very much, chemical control can be avoided some years by mixing rape seed with turnip rape.

Effect of sowing density of oilseed rape on the abundance and within-plant distribution of *Psylliodes chrysocephala*

Nuss H & Ulber B

Institute of Plant Pathology and Plant Protection, University of Göttingen, Germany. Email:bulber@gwdg.de

E-04

Abstract: The effect of plant density and plant architecture on infestation of oilseed rape by cabbage stem flea beetle, *Psylliodes chrysocephala* L., was investigated by using various sowing rates (30, 60 and 90 seeds/m²) in randomized field plot experiments in the autumn of 1999, 2000 and 2001. The lowest sowing rate resulted in larger oilseed rape plants with significantly increased numbers of leaves and lateral buds. The number of *P. chrysocephala* eggs/m² as well as the number of larvae/m² was reduced with decreasing number of plants/m². In contrast, the number of larvae per plant was significantly increased at low plant densities. The within-plant distribution of the larvae was only affected in the year 2000 when vigorous growth of the hybrid oilseed rape cultivar in the autumn enabled an early development of lateral buds particularly at the lowest plant density. Under these conditions, at higher sowing rates (60 seeds/m², 90 seeds/m²) the number of larvae tunneling within the terminal buds was significantly higher than at the low sowing rate (30 seeds/m²) which obviously gave rise to plants providing a sufficient food resource for the larvae within petioles and lateral buds. These results indicate that the most damaging infestation of the terminal buds by cabbage stem flea beetle which often results in considerable overwinter mortality of plants can be reduced by growing oilseed rape at lower plant densities.

Means to control pests in organic oilseed rape production

W. BÜCHS, K. KATZUR

Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection in Field Crops and Grassland, Messeweg 22, 38108 Braunschweig, Germany

E-05

Abstract: Aim of the field experiments was to practice known husbandry practices to control pests in winter oilseed rape for further development. In oilseed rape considerable damages and harvest losses can be caused by the cabbage stem flea beetle (*Psylliodes chrysocephala*), the pollen beelte (*Meligethes* spp.), the cabbage stem weevil (*Ceutorhynchus pallidactylus*), the oilseed rape stem weevil (*C. napi*), the cabbage seed weevil and the brassica pod midge (*Dasineura brasicae*). A farming system that integrates means to promote natural enemies (mulching, seed rate 3,5 kg/ha; field margin weed strips, trap crop strips and drilling in a wide row (50 cm) to use hoeing machine in autumn and spring = **mulching / hoeing**-treatment) was compared to a standard system (ploughing, conventional drilling technique, currying in autumn and spring, seed rate 5.0 kg/ha without weed-strips and trap crop strips = **ploughing/currying**-treatment). The third system was a **mulching/currying**-treatment (seed rate 5kg/ha, inclusive weed- and trap crop strips).

Results

- An acceptable yield level was achieved. In the ploughed treatment the yield was 2.65 t/ha and thus, 20% higher than in the mulching treatments.
- Trap crop strips (cultivar „Express“) which were installed in the treatments with **mulching** showed (compared to field edges without trap crop strips) a higher infestation by the brassicae pod midge, the cabbage seed weevil and the pollen beetle than the crop stand within the field (cultivar „Oase“). For the cabbage stem weevil however no significant differences were recorded
- While the brassica pod midge achieved the highest reproduction rate within the **mulching/hoeing** treatment, for all other pests the highest reproduction rate was recorded in the **ploughing/currying**-treatment..
- The highest abundance (activity density) of predators active on the soil surface were recorded in the mul**ching/currying**-treatment Obviously caused by a higher abundance of natural enemies the reproduction rate of all pest species (except the pollen beetle) was the lowest in the **mulching/currying-**treatment. That means, in this treatment considerably more larvae had to be invested to produce one mature individual of the new pest generation..

All treatment showed advantages and disadvantages as well: the **ploughing/currying**-treatment achieved the highest yield, but also the highest hatching rates of pests of the new generation (except the brassica pod midge). The **mulching/currying**-treat promoted natural enemies, but there is a considerable higher risk of yield losses by weeds. In the **mulching/hoeing**-treatment was the reduction of the weed risk followed by a reduction of natural enemies. Furthermore in this treatment a higher infestation by the brassicae pod midge and other occasional pests occuring in autumn was recorded.

This one-year field trial demonstrated very clearly that organic oilseed rape cultivation is principally possible without larger problems if relevant husbandry practices are considered and used in the appropriate way.

Occurrence in the field and behavioural responses to odours of different growth stages of oilseed rape of Ichneumonid parasitoids of pollen beetles

M. Jönsson

Department of Crop Science, Swedish University of Agricultural Sciences, Box 44, 230 53 Alnarp, Sweden

Abstract: The three most abundant Ichneumonid parasitoid species attacking pollen beetles in southern Sweden are *Phradis interstitialis*, *P. morionellus* and *Tersilochus heterocerus*. The relative abundances of females of these species have been evaluated during two seasons in an area with both winter and spring rape fields. In the beginning of the season *P. interstitialis* was the dominating species, while later, during flowering of the spring rape, *P. morionellus* was the most abundant species. *T. heterocerus* was found in smaller numbers throughout the first season, but were more abundant the second season.

As a part of a study on stimuli affecting the behaviours of females of these parasitoids the responses to oilseed rape odours and to visual stimuli were studied in Y-tube dual choice tests. The parasitoids were offered odours from different growth stages of oilseed rape alone and in combination with green and yellow colours. These tests showed that the two *Phradis* species preferred the odours of oilseed rape in the bud stage when tested against clean air as well as against odours of oilseed rape in the flowering stage. These preferences can be shifted when the odours are combined with yellow and green colours. Females of the third species *T. heterocerus* behaved differently. In this species were a preference observed for both rape in bud stage as well as for rape in flowering stage, although strongest for flowering rape.

E-06

Phenology and spatial distributions of *Dasineura brassicae* and its parasitoids in a crop of winter oilseed rape: implications for integrated pest management.

A. W. Ferguson, J. M. Campbell, D. J. Warner, N. P. Watts, J. E.U. Schmidt and I. H. Williams

Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK

Abstract: The distributions and phenology of *Dasineura brassicae* and two of its parasitoids were studied in relation to the value of spatial and temporal information for the conservation of parasitoids in integrated pest management. Insects were sampled at 40 spatially-referenced points within a crop of winter oilseed rape and the following winter wheat crop. Two generations of *D. brassicae* larvae were collected in water trays as they fell from rape plants in 1999 and insects emerging from *D. brassicae* larval cocoons in the soil were collected in emergence traps pre-diapause (1999) and post-diapause (2000). Spatial distributions were analysed and compared using Spatial Analysis by Distance IndicEs (SADIE). Only 7% of first generation larvae gave rise to emerging insects pre-diapause and 0.2% of first and second generation larvae to emerging insects post-diapause. Parasitoids comprised 42% and 49% of insects emerging from *D. brassicae* cocoons pre-diapause and post-diapause respectively. *Omphale clypealis* was more abundant than *P. subuliformis* in 1999. Pre-diapause, the start of emergence of both parasitoid species was coincident with the emergence of adult *D. brassicae* but the emergence of parasitoids was more prolonged. Emergence of *O. clypealis* post-diapause peaked a month later than either *D. brassicae* or *P. subuliformis*. All insects were markedly edge-distributed and spatially associated pre-diapause but only *O. clypealis* remained edge-distributed post-diapause. The implications for conservation management of parasitoids are discussed.

E-07

Verification of protective sowing ability to concentrate insect pests and their parasitoids around rapeseed field

D. Nerad [(1)], P. Baranyk[(2)]

[(1)] *Union of Oilseeds Growers and Processors (SPZO),* [(2)] *Czech University of Agricultural Sciences Prague*

Abstract: The increase of winter oilseed rape (*Brassica napus*, L.) fields in the Czech Republic caused the higher occurrence of insect pests. The Stem Weevils (*Ceutorhynchus napi*, *Ceutorhynchus pallidactylus*), the Pollen Beetle (*Meligethes aeneus*), the Cabbage Seed Weevil (*Ceutorhynchus assimilis*) and the Brassica Pod Midge (*Dasineura brassicae*) settle down in the plants during spring period and are included in the cardinal insect pests. Some of them do not attack the whole oilseed rape field evenly but prefer especially a margin of the field. The reduction of insect pest population due to the increasing distance from the field margin is called "margin effect".

The lines of plant attractants against insect pests were sown around the margins of oilseed rape fields. Turnip rape (*Brassica rapa* L.), variety Rex, an extra early variety of oilseed rape – Prestol and mixture of Turnip rape, Prestol and spring oilseed rape were used as a plant attractant. The frequency of insect pests on the stem tops of 100 plants (*Meligethes aeneus, Ceutorhynchus assimilis*) was observed in protective sowing and in different distances on the “cross-section” of experimental field. In addition, the percentage of invaded stems and the level of damage (*Ceutorhynchus napi, Ceutorhynchus pallidactylus*) were also calculated. Moreover, the occurrence of pest’s natural enemies and levels of their parasitism were evaluated.

The observed results of the work show, that the first flight of *M. aeneus* could be considerably trapped by a protective sowing. The protective sowing is also very attractive for *C. assimilis*. The number of beetles was the highest in the protective sowing in all years. The attractivity could be partly suggested also for *D. brassicae*. The results prove that the *M. aeneus* and *C. assimilis* are more attracted by turnip rape plants than by early varieties of oilseed rape. The influence of protective sowing on the field infestation by *C. napi* and *C. pallidactylus* was not proved.
The method of protective sowings could contribute to the increase of insect pest parasitoids as well. Detected were mostly the adult endoparasitoids from *Tersilochus* spp. (Hymenoptera: Ichneumonidae). Statistically a significant higher number of the parasitoids in the protective sowing than in the growth behind was prooved. Based on the analysis of the *M. aeneus* larvae, it could be stated, that the level of parasitism through *Tersilochus spp.* ranged between 60 – 100 % in the growth and 75 – 100 % in the protective sowing. There was an evident dependence between the high density of the *M. aeneus* beetles and parallel high density of their parasitoids and the level of parasitised larvae. The level of parasitization inside the field (50 and more meters from the field margins) was lower nearly without differences.

E-08

Rearing and identification of the larval parasitoids of *Psylliodes chrysocephala* and *Ceutorhynchus pallidactylus* from field-collected specimens

H. Barari, S. M. Cook and I. 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 adult parasitoids.

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.

E-09

Incidence of larval parasitism of *Psylliodes chrysocephala* within oilseed rape crops in Germany

B. Ulber & R. Wedemeyer

Institute of Plant Pathology and Plant Protection, University of Göttingen, Germany. Email:bulber@gwdg.de

Abstract: The parasitism of cabbage stem flea beetle, *Psylliodes chrysocephala* (L.) was investigated in various oilseed rape crops in Northern Germany in 1999 to 2003. The most predominant parasitoid attacking the larvae in spring was identified as *Tersilochus microgaster* (Szépligeti) (Ichneumonidae: Hym.), a solitary univoltine koinobiont endoparasitoid. The phenology of emergence and the immigration of adult *T. microgaster* into new oilseed crops was monitored by emergence traps, yellow water traps and Malaise traps. Peak emergence of overwintered adults was observed in mid March or April in fields grown with oilseed rape in the previous year. Female parasitoids were captured in new rape crops in March and April, indicating a high level of synchrony between immigration of parasitoids with the appearance of larval instars of *P. chrysocephala* in petioles.

To study the level of parasitism of *P. chrysocephala* by *T. microgaster,* host larvae were sampled from petioles and stems of oilseed rape plants in March to May. The parasitism was determined by dissecting the larvae. To obtain the adult parasitoids sub-samples of host larvae were reared to pupation and emerging adult parasitoids identified in the laboratory. Percentage parasitism in various years ranged between 24.6 % and 44.4 %. Superparasitism occurred regularly, with up to nine encapsulated eggs or larvae of *T. microgaster* per individual host larva. The effect of host plant architecture and host plant density as well as within-plant distribution and instar of host larvae on larval parasitism by *T. microgaster* was studied in field experiments.

Parasitism of *P. chrysocephala* larvae by *Aneuclis melanarius* (Holmgren) (Ichneumonidae, Hym.) in autumn was observed only in one year of study. Ectoparasitism of larvae by *Trichomalus lucidus* (Walker) (Pteromalidae, Hym.) was found only on isolated host specimen.

E-10

Incidence and feeding activity of epigeic, predatory invertebrates within winter oilseed rape in the UK with comparisons between integrated and conventional crop management.

R. Piper, I.H. Williams

Rothamsted Research, Harpenden, Hertfordshire, UK, AL5 2JQ

Abstract: The EU-funded project MASTER (MAnagement STrategies for European Rape pests) aims to realise the potential of predatory epigeic invertebrates in controlling six major pests of winter oil seed rape. This requires knowledge of the diversity of the predators and their diets. Pitfall trapping was used to assess the incidence of Carabidae, Staphylinidae and Araneae in integrated (ICM) and conventional (STN) oil seed rape plots. The activity density and diversity of all taxa was greater in the ICM plot. Gut dissections revealed that the larvae of *Meligethes aeneus* were eaten in the field by three species of carabid, of which the most abundant was *Nebria brevicollis*. There was also some evidence of predation on *Ceutorhynchus* sp. larvae and adults, although this was rare. The majority of Carabidae and all the Staphylinidae contained only liquid in their crops and Araneae are obligate liquid feeders; therefore a technique other than gut dissections is needed to further define the diet of these species in the field.

Approaches to assess the importance of carnivorous beetles as predators of oilseed rape pests

O. Schlein, W. Büchs

Federal Biological Research Centre for Agriculture and Forestry, Institute for Plant Protection in Field Crops and Grassland, Messeweg 22, 38108 Braunschweig, Germany

Abstract: The feeding ecology and efficacy of common carnivorous beetles from the families Carabidae and Staphylinidae as predators of the most important oilseed rape pest species are currently investigated within the EU-project "MASTER" ("Integrated pest management strategies incorporating bio-control for European oilseed rape pests"). Using several experimental and analytic approaches, significant differences in feeding acceptance, food preferences and mean consumption rates as well as in the degree of partial granivory of eight key predator species which proved to be dominant on winter oilseed rape fields in Braunschweig, Germany (Carabidae: *Poecilus cupreus, Pterostichus melanarius, Nebria brevicollis, Anchomenus dorsalis, Amara similata* and *Pseudoophonus rufipes*; Staphylinidae: *Tachyporus hypnorum* and *Philonthus cognatus*) have been revealed by our laboratory research so far.
Behavioural experiments (non-choice as well as choice feeding trials), biochemical efforts (proving DNA of the rape pest larvae within the predators gut content using PCR after different time intervals of digestion) and microscopic analyses of the predators digestive tract (identifying special morphologic features of the pest larvae within the gut content) have been carried out to the present date. Selected results are presented and the different laboratory approaches are discussed. Considering these findings, a comparison between the different beetle species according to their efficacy as oilseed rape pest predators as well as a provisional assessment of their importance in oilseed rape plant protection can be given.

Impact of predators on pollen beetle *Meligthes aeneus* on rapeseed in Finland

H. M.T. Hokkanen

Laboratory of Applied Zoology, Box 27, Viikki C FIN-00014 University of Helsinki, Finland, heikki.hokkanen@helsinki.fi

Studies on pollen beetle *Meligethes aeneus* predators were carried out in Finland in turnip rapeseed fields first in 1983-84, and later in 1993 and in 1998-2000. Season-long pitfall trapping was the main survey method, but yellow water traps were also used; in addition, sweep netting every two hours for day and night (over two nights) was conducted in 1983, and 15 rapeseed plants were smeared with Tanglefoot insect glue to monitor climbing predators. In 1993 predators were studied as part of an intercropping trial, and in 1998-2000 as part of a biopesticide non-target impact trial; all studies were carried out on spring turnip rapeseed fields. Additionally specific predator exclusion and enrichment studies were conducted over two years in 1984 and 1985.

The most common and abundant predators on rapeseed fields were the carabids *Pterostichus niger* and *P. melanarius*. Feeding trials showed they they also eagerly consume pollen beetle larvae. Other abundant carabid species were *Trechus discus*, *Patrobus atrorufus*, and the families *Amara, Bembidion, Harpalus, Clivina, Calathus* and *Agonum*. Overall, some 40 species of carabid beetles were collected and identified; almost twice that many species are known to occur in the arable fields at the Helsinki University experimental farm. Staphylinid beetles were much less common, only about one-fifth of the number of carabid beetles were caught in the pitfall traps in 1983-84, but about one-half in 1993. Spiders were abundant, and were collected in the pitfall traps at about similar numbers as Staphylinid beetles. Other predators were occasional, and included some bugs (*Nabis ferus* and *Anthocoris nemorum*), few chrysopid larvae, and some coccinellid beetles. In 1988, however, ladybeetle populations in Finland exploded due to an extreme aphid abundance on cereals and on birch-trees, and towards the end of flowering all rapeseed fields that we surveyed were full of ladybeetles. I counted repeatedly about 40-50 beetles per square meter, throughout the fields. In that year, ladybeetle predation on pollen beetle larvae was certainly significant. The most abundant species was *Coccinella 7-punctata*, followed by *Adalia 2-punctata*.

Exclusion trials indicated that the resident predator guild exerts only minimal pressure on the pollen beetle population: the difference between exclusion treatment and control was not significant. The likely reason is that usually carabid activity is at the minimum in Finland at the time of pollen beetle pupation, around early to mid-July. Also the intercropping (rapeseed-clover) trial revealed little differences in predator activity densities between the monocrop and the intercrop, but as the host plant density and the corresponding pollen beetle population per surface area was lower in the intercrop, a significantly higher proportion of the pollen beetle larvae were eaten in the intercrop as compared with the monocrop (i.e., predation pressure pre larva was higher).

Travel details to/from Rothamsted

It would be useful to the organisers and the accommodation staff if we had some idea of your flight/travel plans. Please email neal.evans@bbsrc.ac.uk when you know this information. We will then be able to advise you as the best mode of transport from your arrival point to Rothamsted and may be able to help with local transport. However, the following text provides some extra information:

London Luton airport
The easiest airport to get from/to is London Luton (about 15-20 minutes away). The easiest way to get to Harpenden is by Taxi, especially if there is more than one of you to share the cab. Alternatively, take the free shuttle bus to the Luton Airport Parkway Train Station and catch a London-bound train, checking that the train stops at Harpenden. (It is only 1 stop).

London Stansted Airport
From Stansted, (depending on the time of your flight) the easiest way to Rothamsted is by coach (National Express, http://www.nationalexpress.com/). Bus 777 is direct between Stansted and Luton and takes about 2 hours each way. From Luton Bus Station, the Luton Town Train Station is next door. It is then easy to take the 10 minute train ride to Harpenden (2 stops). (*Please note, for return trips, it is not easy to get to Stansted early in the morning for an early flight*). The other way to/from Stansted is by train into London, change stations and then back out again.

London Heathrow Airport
From Heathrow, the easiest way is for us to book you a Harpenden taxi (this cost about £40 each way). This is particularly true if there is more than one of you arriving at the same time. The cheaper option is to get the London Underground (Picadilly Line) from Heathrow to Kings Cross/St Pancras (~1 hour), then walk the 5 minutes through the subway to Kings Cross Thameslink Station and get a train north (Luton/Bedford via St Albans) to Harpenden (~25 minutes by fast train, **check that the train stops at Harpenden**). Rothamsted Research is the 10 minutes walk from the station (See map) or short taxi ride.

Arrival at Harpenden Station/Local travel during IOBC meeting
All accommodation is within walking distance of Rothamsted and the train station. The Milton Hotel and Mrs Brannigans B&B at 8 Carlton Bank are both about 2-3 minutes walk from the train station. Hall Barn is a little further away (800m), so you may want to consider a taxi if you have a lot of luggage. Rothamsted Manor residents will definitely need to take a taxi (although if you pre-arrange with Neal Evans, we may be able to pick you up depending on time of arrival). The rest of the time, everywhere is within walking distance. However, the weather can be inclement at this time of year, so it is a good idea to bring an umbrella.

Accommodation map HARPENDEN