IOBC-WPRS

Working Group "Integrated Control in Oilseed Crops"

OILB-SROP

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

Proceedings of the meeting

at

FIAP, Paris (France)

29th September-1st October, 2008

Edited by
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Neal Evans and Bernd Ulber

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

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Preface

At our last meeting, held in 2005 in Poznan, Poland, we decided to meet next in Paris, in 2007. This meeting was postponed one year due to schedule difficulties and we met at the end of September 2008 at FIAP Jean Monnet in the center of Paris. The meeting was very well organised by colleagues at CETIOM and brilliantly co-ordinated by Xavier Pinochet; we gratefully acknowledge the input of everyone involved. It was a very pleasant stay in Paris; we enjoyed an impressive city tour and a very tasty (and amusing!) conference dinner – a pity for those who were absent. This was especially true for Sam, who became mother just a short time before the meeting and so was unable to attend. However, we all enjoyed her greeting slide, presenting little Emily at only a few days old. A good excuse for absence!

The working group meeting was a bit smaller than the previous one in Poznan. However, 54 participants from 14 countries attended and 54 papers were presented, including 32 oral presentations. These are now presented in this bulletin. We apologize for the delay in publication. This was mainly attributed to a set of private problems, including the birth of Birger's youngest son, altogether resulting in a total loss of disposable time for both Sam and Birger. However, we finally managed the editing task with the help of Neal Evans and Bernd Ulber. We are very grateful for their assistance and hope that you agree that in this case 'good things come to those who wait!'

During the meeting a convenor election was conducted because Birger had led the group for 5 years and statutes of IOBC prescribe new elections at 5 year intervals. This election was managed by Barbara Ekbom and Xavier Pinochet with the result of Birger's re-election. This was mainly attributed to the situation that there was no other nomination (!). However, Birger agreed to take the responsibility for another 5 year-term.

During the meeting we also considered the venue for the next meeting. Bernd Ulber offered to organize the meeting in 2011 in Göttingen, Germany. This suggestion was accepted by the participants. Therefore, we hope to meet you all again in Göttingen!

Eine gute Reise und auf Wiedersehen in der Universitätsstadt Göttingen!

Birger Koopmann and Sam Cook

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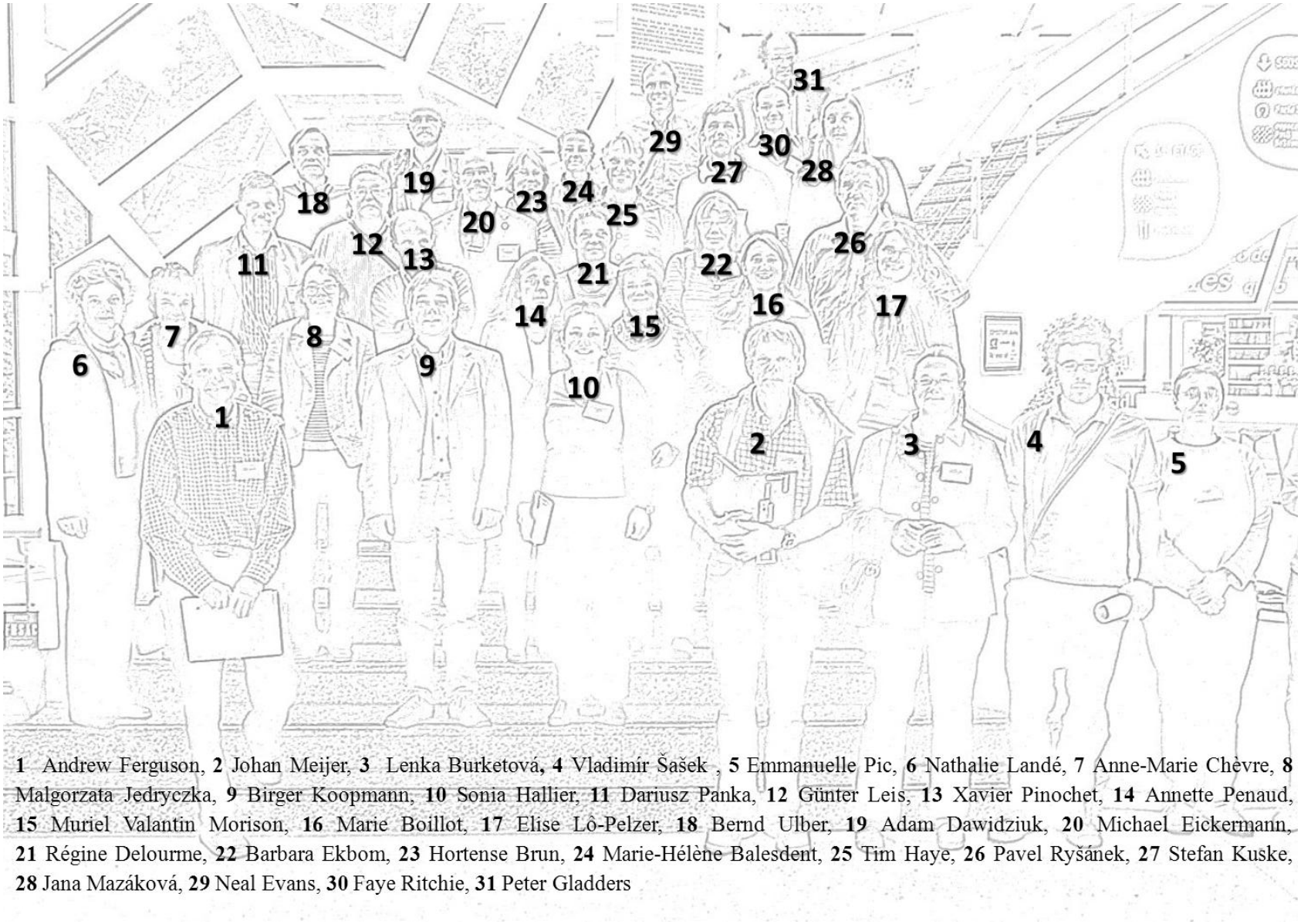

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European network for the durable exploitation of crop protection strategies (ENDURE)

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Abstract: The overall objective of this Network of Excellence (acronym ENDURE) is to reshape European research and development on pesticide use in crops and establish Europe as a leader in the development and implementation of sustainable pest management strategies. ENDURE aims to create a coordinated structure that takes advantage of alternative technologies, build on advances and complementary expertise in agricultural sciences, ecology, behaviour, genetics, economics and social sciences, and connect researchers to other stakeholders in extension, industry, policy-making and society at large. This multi-disciplinary and cross-sector approach is designed to foster the development and implementation of strategies rationalising and reducing pesticide inputs as well as reducing the associated risks. The specific goals of ENDURE are to integrate research capacity and resources currently fragmented across Europe, to enhance the research-to-R&D innovation process by creating working relationships between researchers and practitioners in extension and farming, to engage with industry, policy-makers and civil society to help define the research agenda, and to pass on knowledge, know-how and resources through training, education, and dissemination.

Key words: pests, diseases, weeds, integrated control, system case studies

Introduction

The ENDURE Network of Excellence brings together more than 300 researchers in the fields of agronomy, biology, ecology, economics and the social sciences from 18 organisations in 10 European countries. The project is funded for four years (2007-2010) by the European Commission's Sixth Framework Programme, priority 5: 'Food Quality and Security'. The network's objectives are to:

1) build a lasting crop protection research community.
2) Provide end-users with a broad range of short-term solutions to specific problems.
3) Develop a holistic and systems-based approach to sustainable pest management.
4) Take stock of and inform plant protection policy changes.

Four integrating themes

Identifying short-term solutions

Farmers facing new constraints and challenges to pest management require new solutions. Some of these solutions are achievable in the short term, allowing farmers to respond to the new demands placed on them while remaining competitive. Significant short-term progress on optimising and reducing pesticide use in existing farming systems can be made through better use of current knowledge and resources to improve farming practices. Within ENDURE, crop-specific case-studies are exploring the feasibility of changing farmer practices and translating research findings into practical options. The case studies underway or already completed

address pest problems in wheat, apple and pear, tomato, potato, banana, field vegetables, grapevine, and maize, in addition to integrated weed management.

Strategies developed in a local context are being compared to investigate their relative advantages, risks and cost-effectiveness, as well as their potential for being implemented more broadly at the European level.

Introducing innovative strategies and reducing cropping system reliance on pesticides

In the longer-term, new technologies and approaches are required to supplement those already available for reducing over-dependence on pesticides. In accordance with the concept of integrated pest management (IPM), ENDURE is examining ways to integrate new developments in modelling, plant resistance, biological control, decision support, ecology, socio-economics and agronomy to design agricultural systems that can be implemented at a landscape scale and which minimise risks of pest adapting to individual control tactics.

To ensure sustainability, special emphasis is given to multi-criteria assessment tools that encompass environmental and economic impacts as well as the effectiveness of crop protection methods in suppressing pest incidence and abundance.

Assembling a permanent research community

The goal of a Network of Excellence such as ENDURE is to create a community of researchers with expertise in the full range of disciplines needed to develop and promote a more coherent framework for IPM. In this respect, the network includes a number of activities aimed at identifying critical knowledge gaps, and generating a common research agenda consistent with future needs for crop protection in the EU. To assist with these objectives, the network has recently completed a wide-ranging foresight study analysing the pressures and conflicting demands likely to be placed on agriculture over the next 20 years. This study has produced five contrasting scenarios resulting from different combinations of political, economic, environmental and societal drivers. These scenarios have since been presented to a wide range of audiences to fine-tune their design and validate their contrasting demands for research on crop protection.

Another important component of ENDURE is termed the Virtual Laboratory (http://www.endure-network.eu/virtual_laboratory; Figure 1), providing access to a range of knowledge, resources and tools for the research community as a whole. The Virtual Laboratory also contains specific research platforms for topics of high priority within crop protection research such as the EuroWheat platform which focuses on control of foliar cereal diseases (www.eurowheat.org; Figure 2).

Interaction between members has been further strengthened by a programme supporting reciprocal working visits between partner organisations by young or established scientists and research students. This mobility programme has so far funded 48 exchange visits that have led to new training opportunities and fostered greater collaboration between nationally-funded research projects.

To ensure that the information collected and new results produced by ENDURE are extended to end-users, ENDURE has created a unique dissemination tool called the ENDURE Information Centre (EIC; Figure 3). The EIC draws on expertise from across Europe including all aspects of crop protection research. It aims to cover all major cropping systems and include the recommendations of research done within the network. The EIC will also disseminate more general information on pest incidence, perceived new threats to European agriculture and progress with research on new control tactics.

Figure 1. Screen shot (greyscale) of the front page of the ENDURE Virtual laboratory showing different "rooms" housing information on resources, facilities and expertise to facilitate collaborative research between ENDURE scientists.

Informing policy makers

The EU is currently contending with important new legislation targeted at reducing pesticide use and promoting moves towards greater implementation of IPM in member states. Specific responses to this legislation are likely to vary considerably between countries, but there are also several generic challenges such as the greater use of biological control on field crops, sustaining the effectiveness of pest-resistant crop cultivars, and issues related to forecasting, pest thresholds and reliable decision support. ENDURE has a commitment to providing scientific support in response to such challenges, and to facilitating the implementation of Directives such as the Thematic Strategy for sustainable pesticide use. Analysis of drivers and barriers to IPM implementation is one step towards helping policy-makers in the different member states to develop their own action plans.

Current stage of the network and future plans

The four-year funding of the network by the EU ends in December 2010. Throughout 2010 the main emphasis will be on publishing results and outputs as scientific papers and technical or popular leaflets, and on transferring new tools, methods and information to the Virtual Laboratory or the EIC. Discussions are taking place on possible mechanisms for maintaining the partnership beyond this period and for incorporating other organisations into the ENDURE community. Plans for the future will be finalised and presented at an international conference to be hosted by the network in Paris in November.

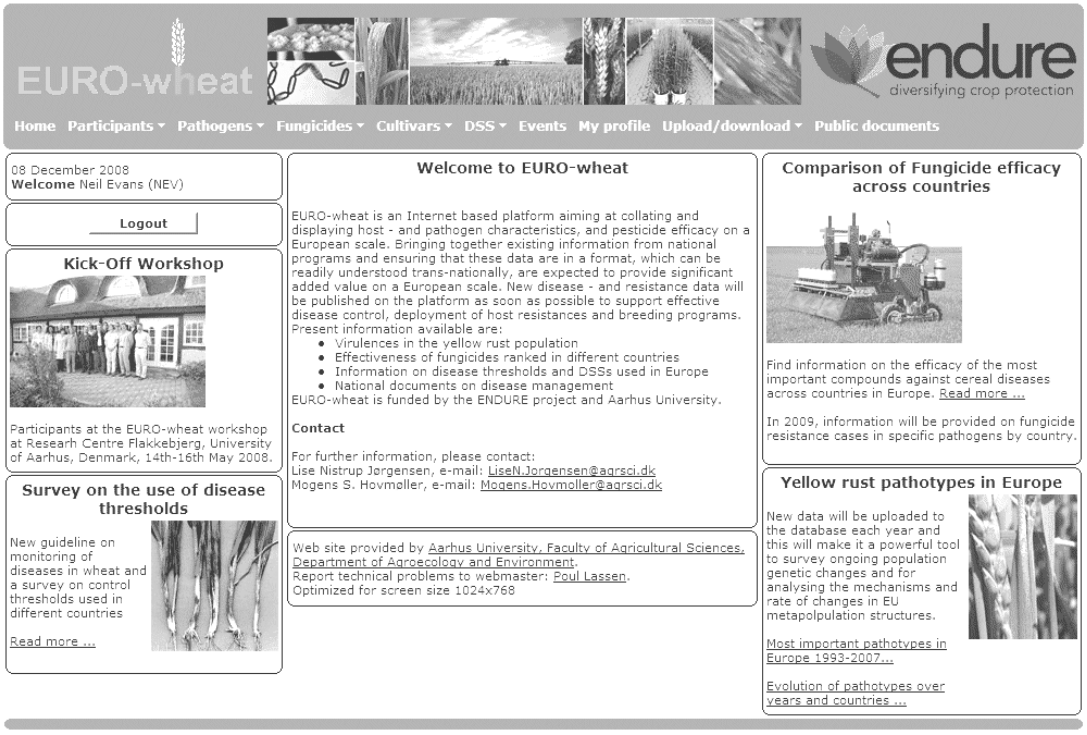

Figure 2. Screen shot (greyscale) of the front page of the EuroWheat platform (www.eurowheat.org) within the ENDURE Virtual Laboratory with useful information for scientists, cereal producers and their advisors.

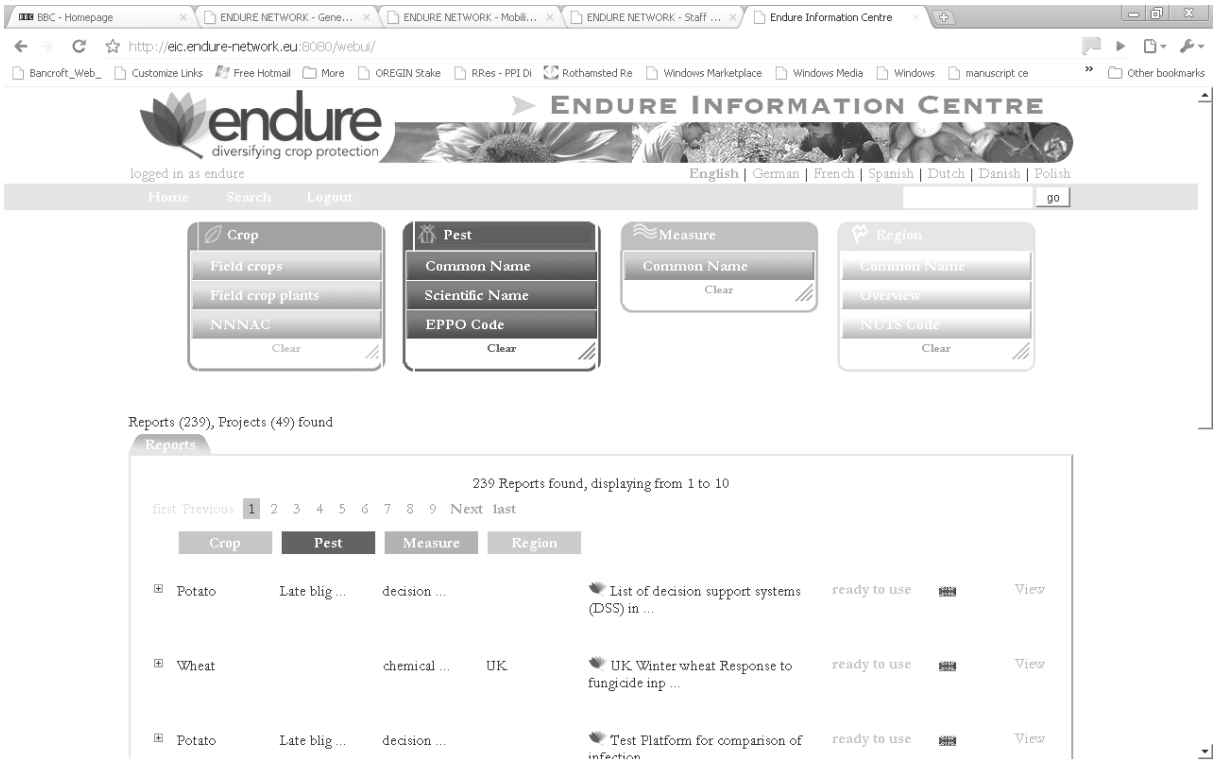

Figure 3. Screen shot (greyscale) of the main search page of the ENDURE Information Centre (EIC) which provides farm advisors and growers with up-to-date technical information.

Integrated Control in Oilseed Crops
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Utilization of a model to re-design integrated crop management for winter oilseed rape

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Abstract: Concerns about the adverse impacts of pesticides on the environment and their inevitable negative side-effects on non-target organisms have been growing since the 1960's. As a consequence, regulatory bodies take into account the environmental effects of pesticide applications, leading to increased restrictions in their use or to their revocation or banning (van der Werf, 1996). For winter oilseed rape (*Brassica napus* L.) in France, herbicides, fungicides and insecticides constitute the largest component of variable operating costs: representing 20% of the gross margin. On the other hand, in order to avoid disease occurrence, weed infestation and pest attack, diverse elementary control methods (genetic, cultural, mechanical, chemical) can be used in integrated pest management strategies (Dhaliwal *et al.*, 2004, El Titi *et al.*, 1993). Since each technical operation is likely to modify the sanitary status of a crop, it is therefore possible to design cropping systems to minimize crop loss induced by pest population with a limited use of chemical control. Because of the complexity of the considered systems due to interactions between cultural practices, crop status, soil, climate and pest populations, modelling is a key tool to propose innovative cropping systems less vulnerable to pests. Several experiments (1994-2004) have been used to analyze and simulate (i) the effects of agricultural practices on the pests and their interaction with the crop and (ii) the yield losses induced by pests. A bio-economic model, named OMEGA sys, has been developed in order to represent the effects of crop management either on crop yield, weed biomass and stem cancer attacks. The first aim of this model is to help in building environmental friendly crop management systems. The inputs of the model are climatic variables and the combination of each element of crop management, while the outputs are potential, attainable yield, number of pesticide treatments and gross margin. An algorithm of gross margin optimization is combined to the agronomic model in order to rank the diverse combinations of crop practices. This paper makes a short description of the OMEGA sys, gives some results of assessments and illustrates the new integrated crop management strategies than can be obtained with such methodology in two agronomical contexts and with two weed/disease pressures. Depending of the economic scenario, the first crop management strategy selected by the OMEGA sys are characterized by no or few pesticides utilization. The best crop management systems selected are different between the different agronomic context (soil, preceding crop and weed pressure).

Key words: Winter oil seed rape, integrated crop management, *Phoma* stem canker, weed, bioeconomic model, environmental assessment

Introduction

Winter oilseed rape (WOSR) (*Brassica napus* L.) is widely used in French farming systems ($1.5\ 10^6$ ha, CETIOM) and is also widely present in many other European countries. Due to the increasing demand in energy and increasing value, this crop is now cultivated in new regions of France such as the west. This crop is of potential value in terms of market requirements and agronomic potential. Its oil is particularly beneficial for human health as it contains omega 3 fatty acids (Renaud, 1996, Bourre, 1996).

However, this crop is known to be very dependent on pesticides: it is attacked by numerous diseases, pests and weeds (Alford *et al*., 2003), which are difficult to control without chemical treatments. No extensive study has investigated the effects of crop management on WOSR in low input systems, accounting for the current lack of pesticide-free crop protection strategies for this crop. For WOSR in France, the cost of pesticide treatment is the major component of the farmer's total variable operating costs (almost 20%).

Concerns about the adverse impacts of pesticides on the environment have increased since the 1960's. Nowadays, there is a great need to reduce the structural dependence on pesticides of intensive agricultural productions world-wide. As a consequence, policy makers have strengthened regulations on pesticides (van der Werf, 1996) and farmers are now encouraged to minimise pesticide use, particularly for weed control (Lutman *et al*., 2000). For winter oilseed rape (*Brassica napus* L.) in French conventional systems, the cost of herbicide treatment is the major component of the total farmer's variable operating costs (Primot *et al*., 2005). Pre-plant incorporated and/or pre-emergence herbicides account for 96% of the herbicide treatments

In that context, the development of integrated management has become an important topic in agronomic research. Recent studies (Valantin-Morison *et al.*, 2003 and Valantin-Morison and Meynard, 2008) demonstrated that the main limiting factors of WOSR in organic farming were nitrogen and weeds. But it is also known that some diseases such as stem canker which is considered together with *Sclerotinia* as the most serious diseases of oilseed rape world-wide (Aubertot *et al.*, 2002; Koch *et al.*, 2007). In order to limit the utilisation of pesticides, various control methods could be distinguished: chemical, genetic, physical and cultural control. The latter consists in adapting one or several cultural operations (including cropping sequence) that does not correspond to a chemical, biological, genetic or physical control operation aimed at limiting pest populations. These elementary control methods can be combined to control pest communities through Integrated Pest Management strategies (Dhaliwal *et al.*, 2004). More generally, these methods are the cornerstone of Integrated Crop Management strategies that avoid waste, enhance energy efficiency and minimize pollution (El Titi *et al.*, 1993). However, concerning WOSR, there are very few studies which are aimed at designing new low input or pesticides-free crop management (Dejoux *et al.*, 2003). This is all the more difficult because numerous interactions between crop practices result in complex and sometimes antagonistic effects.

In order to take into account the integrated effect of several crop practices and in order to assess and thereafter to choose the "best crop management" strategy, a model could help. However, to best of our knowledge, models which consider either crop growth/development and pest damage do not exist for WOSR. Some models allow the optimisation of control of disease or weeds through chemical treatments at the field scale: as in the case of Eprire (Zadoks, 1989) for disease or Weedsoft (Neeser *et al.*, 2004) for weeds. These decision support systems have been developed for soybean to help farmers and consultant in order to select weed control strategies (Neeser *et al.*, 2004). Neuhoff *et al.* (2005) have developed another DSS (Wecof) for weed control in organic winter wheat. This tool is expected to

improve weed management and higher revenues for farmers. Recommendations on variety choice are complimented by information on crop spacing, seed quality, fertility management, crop rotation and other factors expected to increase crop competitive ability against weeds. Concerning *Phoma* stem canker, a model named SiPOM (Lo-Pelzer *et al*., 2008) has been developed to simulate the effects of cropping systems and their spatial distribution on blackleg epidemics over years. However, no model exists that takes into account more than one pests' damage to design and assess several low input (or pesticide-free) crop management systems in diverse climates and soil environments.

The purpose of this study is to present a model which has been used to design new crop management systems, with low pesticide use that simulates crop growth, weed invasion risk and *Phoma* stem canker development – and their consequences on yield. This model, named OMEGA sys, is based on experimental results, expert knowledge and information from the literature. An original feature of our model is that the input variables can be easily measured at sowing and in early autumn. Moreover, this model takes into account crop management. This model has been developed to help researchers and advisors in agronomy to explore the economic and environmental consequences of new integrated strategies, based on low inputs.

Material and methods

Description of the model

General frame, inputs and outputs

OMEGA sys is an oilseed rape model to experiment and generate alternative systems. The basic structure of this simulator is composed of an agronomic model and an economic model. The agronomic model is composed of a dynamic plant sub-model and pests' sub-models. The dynamic plant growth sub-model is composed of soil module, plant growth module, and nitrogen absorption module. This crop model was inspired by the Azodyn rape model (Jeuffroy *et al.*, 2001; Valantin-Morison *et al.*, 2004). This crop model is combined with a weed biomass model and a *Phoma* stem canker model, which is based on one of the SIPPOM sub-models (Aubertot *et al.*, 2004b). The economic model is based on the optimization of gross margin obtained by yield for one crop management.

Four categories of input variables are considered in this study, variables related to weed population characteristics: weed species and weed density at the date of weed emergence; variables concerning stem canker: resistance of variety, primary inoculum and variables related to cropping practices: crop sowing date, crop density, soil mineral nitrogen, soil tillage, varietal resistance, fungicides and herbicides and dates of application and nitrogen fertilisation. Finally, variables related to the description of the climate and soil environment: soil characteristics (clay, $CaCO_3$, total N content, bulk density and thickness of the ploughed layer), nature and amount of residues of the previous crop, organic amendments, daily climatic data, N and water content of the soil at the date of harvest of the preceding crop.

The outputs of the agronomic model are three types: (i) potential yield, the yield obtained after pest damage, the oil content; (ii) the amount of mineral N lost by leaching during the growth cycle and the amount of mineral N in the soil at harvest; (iii) pest incidence, weed biomass and disease index. The outputs of the economic model are (i) the gross margin of each crop management tested and (ii) the ranking of crop management tested.

Dynamic plant growth sub-model

The model, described by Jeuffroy *et al.* (2001), simulates daily crop growth, based on intercepted radiation, and reduction in radiation use efficiency and leaf area index, linked with

crop nitrogen status. Crop growth is simulated according to the Monteith's formula, with the parameters from the literature. Effects of N deficiencies on the leaf area index and on the efficiency of radiation interception were included with relationships depending on crop NNI (Colnenne, 2002). The radiation use efficiency was simulated according to air temperature and crop NNI (Justes *et al.*, 2000). Crop N content and NNI were estimated using the relationships determined by Colnenne *et al.* (2002). Leaf fall is simulated after a life duration of 650 degree-days (Dejoux, 2000). The yield reduction, caused by N deficiencies, is simulated according to the crop N status at the beginning of flowering and at G4 stage (Colnenne, 2002). The soil sub-model for oilseed rape was the same as for wheat, based on the balance sheet method. Also, to take into account the possibility of nitrate leaching during winter and the effects of dry periods on N mineralisation, a sub-model was added, simulating the evapotranspiration, the transfer of water and nitrogen between the various layers of the soil; the evolution of the rooting depth along the cycle was also added. The amount of mineral nitrogen from the mineralisation of the fallen leaves is simulated, depending on their C/N ratio, itself linked to the crop N status (characterised by the nitrogen nutrition index, NNI) at the date of the fall (Dejoux, 2000), on the N content of the fallen leaves at the date of the fall, daily air temperature and time from the date of fall.

Weed biomass sub-model

The weed biomass model is not dynamic and is based on predicting models, which have been evaluated in Primot *et al.*, 2005. This model provides the weed biomass at the beginning of winter and thereafter the yield loss due to this weed biomass, accounting for the hypothesis that competition effects are major during the vegetative period of the WOSR growth cycle. This quantitative model is a statistical model and depends on diverse crop management parameters (crop density, soil tillage before sowing, nitrogen availability during vegetative stage) and climatic conditions from crop emergence to the beginning of winter and the type of weeds. The type of weeds at crop emergence is provided by a repartition table of weeds' type, depending on the preceding crop, the soil tillage and the date of sowing. These parameters of weed types have been obtained thanks to experiments made in diverse sites of France and partly published in Valantin-Morison and Meynard, 2008, and in Primot *et al.*, 2005. The pressure of weeds is a variable input.

Stem canker sub-model

This sub-model is based on SimCanker (Aubertot *et al.*, 2004b). It provides the severity of the disease (Disease index or G2) and the yield loss due to infection. G2 is calculated from the maximum number of leaf spots per plant during the infection season, the thermal time for the last trimester and the fresh aerial biomass per surface unit at the beginning of the winter, calculated by the dynamic crop growth sub-model. This DI is used to reduce potential yield, given by the dynamic crop model. The initial level of leaf spot is given by a table of parameter, depending on the choice of date of sowing and nitrogen in soil before sowing. Those parameters have been obtained thanks to experiments in 2001 and 2002 (Aubertot *et al.*, 2004a).

Economic model

The objective of this sub-model is to develop a method of comparison and ranking of several crop managements, based on the optimisation the gross margin and regarding the capability of the model to adapt a decision during the vegetative period.

Assessment

Evaluations of the model were realized by comparing simulations to observations from several field experiments in three different regions (Table 1). Those experiments were not used to adjust parameters of the model. Experiments done in Versailles were solely used to establish the initial table of distribution of weed type, depending on the date of sowing, soil tillage and preceding crop. In those experiments weeds and *Phoma* stem canker were the sole pests observed. For the other pests, utilization of pesticides was permitted to reduce their damage. The observations which have been used are: yield with or without the two pests' damage and the gravity score of *Phoma* stem canker and weed biomass. In order to assess the quantitative performance of the OMEGA sys, we used the root mean square error prediction, *ie* the mean differences between simulated and observed variables.

Table 1: Description of experiments that have been used to assess OMEGA sys

Year of harvest	**Site**	**Type of soil**	**Number of crop managements tested in the trials**	**Cropping practices which have changed in several crop managements**
2005-2007	Yvelines (Versailles)	Deep soil with silt	4 and control strips with pesticides or without any pesticides	Date of sowing, row spacing, nitrogen, plant density, herbicides, mechanical weeding, soil tillage before sowing.
2006	Yvelines (Grignon)	Superficial soils with clay	As Versailles	As Versailles
2005-2007	Normandie (Coudres)	Superficial soils with silt	2 and 1 control strips with pesticide in 2005	Date of sowing, row spacing, plant density, herbicides, mechanical weeding
2006-2007	Picardie (Ham and Heudicourt)	Deep soil with silt	From 2 to 6 – no control strips	Date of sowing, row spacing, plant density, herbicides, mechanical weeding, soil tillage before sowing

We also compared the ranking of the crop management tested in fields, thanks to the ranking test of Spearman (David *et al*., 2005).

Utilization

In order to illustrate the potential use of OMEGA sys, simulations were carried out in 6 situations combining two soil types and depths, two preceding crops and two weed pressures (Table 2). Each modality for each factor (climatic condition, soil condition and cropping practices) was combined with each of the others, which resulted in 128 crop managements tested. Climatic conditions between two different decisions (for example between date of sowing and herbicide spraying) were randomly chosen in 4 successive years, which resulted in 16 climatic hazards for each simulation.

Table 2: Factors that have been used to test OMEGA sys

Techniques	**modality**
Soil conditions	Deep soil with silt and superficial soil with clay
Preceding crop	Grain legume or cereals
Weed pressure	High (100 pl/m²) and low (10 pl/m²)
Price of WOSR and costs of operations	220€ t or 420€ t Cost of nitrogen, ploughing, harrowing and spreading stable as 2 years ago / or increased by 30%
Date of sowing	08/01; 08/30
Soil tillage	Ploughing or not
Nitrogen spread in autumn (kg/ha)	Sugar beet vinasse (0 or 100)
Plant density (pltes/m²)	60-30
Herbicide	Pre emergence or not
Mechanical weeding	1 harrow or not
variety	Low susceptibility (Pollen)
fungicide	1 spray or not

Results and discussion

Assessment

Table 3 describes the root mean square error of the agronomic model of all crop managements tested at the field scale in each region. The error of prediction of potential yield ranges from 0.3 t/ha to 0.67 t/ha and is systematically lower than the error of prediction of yield with cumulated pest damage. However, in Normandie, either in 2004-2005 or in 2005-2006, the prediction of yield with cumulated pest damage was reduced mainly because of the error of prediction of blackleg severity score and weed biomass. Figure 1 confirmed those results.

Utilization of OMEGA sys

The combinations of the several crop practices in 4 different conditions and with two scenarios of prices (Table 2) enabled us to rank 128 crop managements on the gross margin criteria. We isolated the top five for each initial condition (preceding crop,*soil type,*weed pressure) and gathered them into 7 groups (Table 4). These criteria helped us to identify and characterise the main strategies of cropping systems that were selected by OMEGA sys.

Table 3: Root mean square error of predictions

Site	**Yvelines (Versailles)**	**Yvelines (Grignon)**	**Normandie**	**Normandie**	**Picardie**
Year	**2005-2006**	**2005-2006**	**2004-2005**	**2005-2006**	**2005-2006**
Potential yield (t/ha)	0.64	0.3	0.67	No experiment results	
Yield with cumulated pests injuries (t/ha)	0.69	0.42	1.4	0.72	0.78
DI	1.08	0.34	5.3	2.1	2.8
weed biomass ew (t/ha)	0.11	0.006	0.3	0.72	0.09
Number of crop management which have been well ranked	4/8	6/8	5/6	2/2	2/3

Table 4: Classification in global strategies of the first crop managements selected by the model.

Name of crop strategy	Global objective	Sowing date	Soil tillage	Organic fertilisation in autumn	Herbicide	Mechanical weeding	Fungicide
A	Weed smothering/ no weed control/no disease problem	Early date	Ploughing	Yes	No	No	No
B		Normal date	Ploughing	Yes	No	No	No
C	Weed control with harrow/ no disease problem	Normal date	no	Yes	No	harrow	No
D		Normal date	no	No	No	harrow	No
E	Sowing and that's all	Normal date	no	No	No	No	No
F		Normal date	Ploughing	no	No	No	

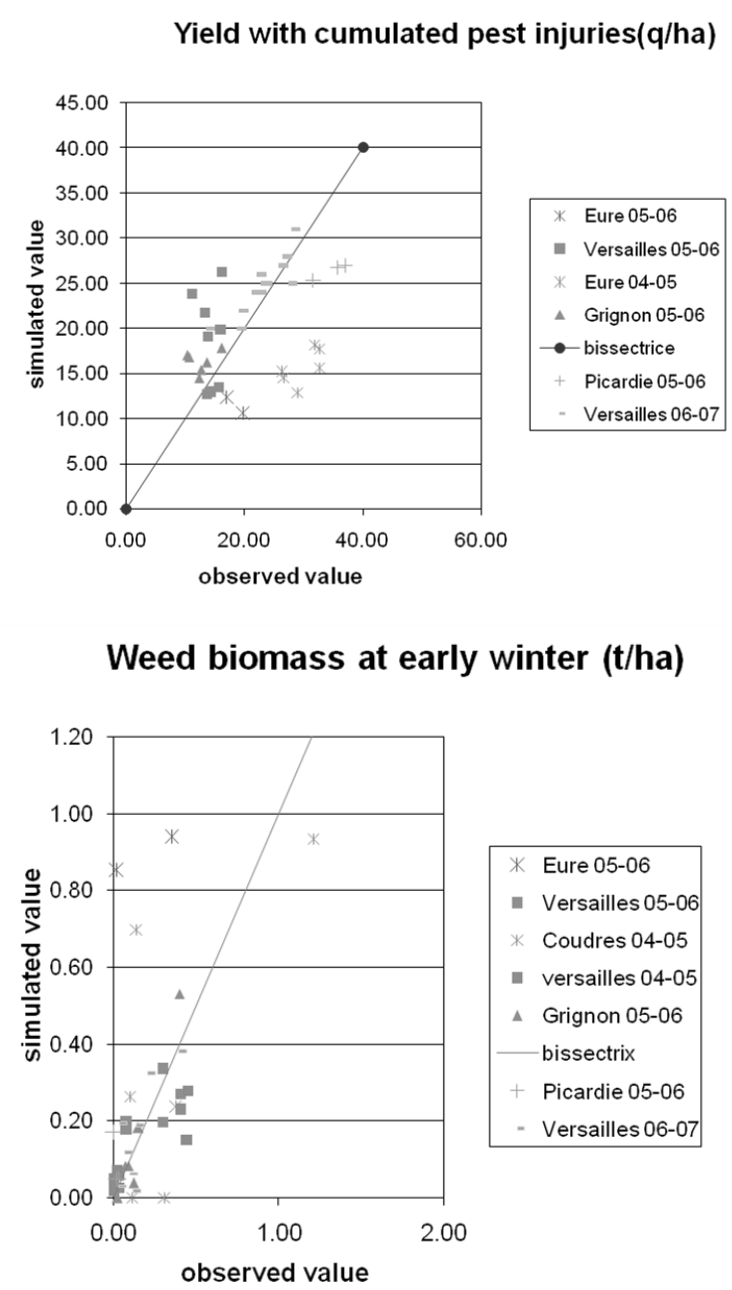

Figure 1: Distribution of simulated and observed values of weed biomass and yield among the different crop management tested

Adapt the optimal choice of crop management in diverse soil conditions and preceding crops

The first result of this analysis is that the optimal strategy differs according to the initial conditions (Table 5). Indeed, comparing the results obtained in the two soil types with the same high pressure of weed and cereals as preceding crop, the major crop managements selected by the model are distributed in different strategies (Table 5). Comparing the results obtained when the two preceding crops are in deep soil with silt with the same high weed pressure, the major crop managements selected by the model are distributed in different strategies (Table 5). When the preceding crop is a grain legume in deep soil with silt, the

global strategy seems to be: reduced soil tillage, no nitrogen during autumn, reduced weed control. This might be explained by the fact that in such situations, the nitrogen in the soil is sufficient to permit the production of high crop biomass quickly after sowing, resulting in the reduction of weed biomass by a smothering effect.

Table 5: Distribution of the five first crop managements selected by OMEGA sys in the 7 crop strategies for the different initial conditions (low prices of WOSR and low costs of operations)

Strategy	Deep soil/**high** weed pressure/ *grain leg*	Deep soil/**high** weed pressure/ *cereals*	Deep soil/**low** weed pressure/ *cereals*	Superficial soil/**high** weed pressure/ *grain leg*	Superficial soil/**high** weed pressure/ *cereals*	Superficial soil/**low** weed pressure/ *cereals*
A	0	1	1	0	0	0
B	0	1	2	0	0	3
C	0	2	0	0	0	0
D	3	1	1	3	3	3
E	0	0	1	0	1	1
F	2	0	0	2	1	1

In conclusion, we can argue that OMEGA sys is able to select the appropriate crop management for one given initial condition. Moreover, the crop management that OMEGA sys selects is characterized by low pesticide use (no chemical herbicides, and no fungicides), which results in combining high gross margin and potentially low environmental impact. This result has also been observed by Rolland *et al.*, 2005, and by Loyce *et al.*, 2006.

Adapt the optimal choice of crop management when economic context is changing

For one of the initial conditions (deep soil with silt and grain legume as a preceding crop, high weed pressure), OMEGA sys has been used with high prices of WOSR and high costs of operations. In such cases, strategies number C and A are retained but in one case, half the amount of fungicide is used, which was not observed for lower prices, as mentioned in the above section. Moreover, the crop managements selected in such economic situations are characterised by the utilisation of organic nitrogen fertilisation in autumn. This might be explained either (i) by the high capability of this crop to absorb nitrogen in soil, all the more that sowing date is early (strategy A), or (ii) by the effect of nitrogen on weed smothering (strategy B), as reported by Dejoux *et al.*, 1999. Consequently, the utilisation of cheap nitrogen during autumn, combined (strategy C) or not (strategy A) with mechanical weeding resulted in no chemical weed control, despite high prices of WOSR and high cost of harrow utilisation.

Find a solution when crop management could result in antagonistic effects

In some cases the choice of cultural control to reduce weed biomass could result in an increase of *Phoma* stem canker; resulting in antagonistic effects of crop management on the

two pests. In such situations, we make the hypothesis that the utilisation of a model such as omega sys will identify the best crop management. Therefore, Table 6 indicates the five first crop managements selected by the model for the different initial conditions, and the values of yield, disease index and weed biomass. In this table, we observe that in the first initial condition, the model selects one crop management (N°12) which is characterised either by low disease index, and low weed biomass and high yield. However, in such a situation, weed pressure is high and organic nitrogen achieves high values, which could have induced high disease index of *Phoma*.

Table 6: Description of yield, disease index and weed biomass of the five first crop managements which OMEGA sys has selected.

Soil and initial weed pressure conditions	**CM number**	**Yield (t/ha)**	**Disease Index (1-9)**	**Weed biomass**
Deep soil with silt/ preceding crop is a **grain legume** High weed pressure (>100 pl/m²)	10	2.8	3.9	0.22
	12	***2.95***	***3.9***	***0.064***
	68	2.6	3.6	0.35
	75	2.9	3.9	0.16
	76	2.9	3.9	0.16
Deep soil with silt/ preceding crop is a **cereal** High weed pressure (>100 pl/m²)	***66***	***2.5***	***3.6***	***0.21***
	69	2.6	5.9	0.21
	70	2.7	5.9	0.21
	72	2.7	5.9	0.19
	78	2.6	6.1	0.04

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Nitrogen fertilisation of winter oilseed rape: impact on insect pests, their parasitoids and plant diseases

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Abstract: Nitrogen fertilisation is essential for maximized oilseed rape yields. However, very little is known about the impact of fertilisation on insect pests and diseases. We studied the impact of additional nitrogen on winter oilseed rape pod number, disease abundance and the oviposition activity of the insect pests *Meligethes aeneus* and *Ceutorhynchus assimilis* and their parasitoids. Insect sampling, pod counts and disease assessments were carried out on plots with seven different N-fertilizer levels (0, 60, 80, 100, 120, 140 and 160 kg/ha). Fertilisation increased significantly the number of pods and decreased the disease scores of the most severe plant disease *Alternaria brassicae.* There was no clear correlation between fertilisation and insect damage. The least preferred plants for *M. aeneus* were in plots with 140 kg/ha and with no additional nitrogen fertilisation. There was no significant difference between plots in the number of damaged pods caused by *C. assimilis*. The parasitation rate of *M. aeneus* was low whereas *C. assimilis* larvae were 100% parasitizised. This study showed that intermediate nitrogen fertilisation did not attract more insect pests but was favourable to parasitoids and might even reduce disease problems, such as dark pod spot.

Key words: winter oilseed rape, nitrogen fertilisation, *Meligethes aeneus*, *Ceutorhynchus assimilis*, diseases, parasitoids

Introduction

Winter oilseed rape (*Brassica napus* L.) is the most important oilseed crop in northern Europe. Although the cropping of spring oilseed rape is prevailing in Estonia, recent mild winters have encouraged farmers to enlarge the growing area of winter oilseed rape. Oilseed rape is known to require high amounts of nitrogen (N), therefore mineral fertilisation is essential for maximized yields. It has been previously shown that variation in seed yield and yield components are influenced by availability of N during the growth and development of winter oilseed rape plants (Scott *et al.*, 1973; Rathke *et al.*, 2006). For economical and ecological reasons, exessive nitrogen fertilisation is a crucial issue, thus external N-input should meet the optimal demand of the plant to maximize yield and to avoid subsequent N-leaching from the soil. However, little is known about the effect of mineral nitrogen fertilisation on the occurrence of diseases and the impact on the abundance of insect pests and their parasitoids is not reported (Rathke *et al.*, 2006).

The major insect pests of oilseed rape include pollen beetle (*Meligethes aeneus* Fab.) and cabbage seed weevil (*Ceutorhynchus assimilis* Payk.). In Estonia, the latter is phenologically better synchronised with winter than spring-sown oilseed rape (Veromann *et al.*, 2006a). The abundance of insect pests can be efficiently controlled by their parasitoids (Walters *et al.*, 2003). Previous studies have shown that major species parasitizing *M. aeneus* are *Diospilus capito* (Nees), *Tersilochus heterocerus* (Thomson) and *Phradis morionellus* (Holmgren); the

main parasitoids of *C. assimilis* are *Trichomalus perfectus* (Walker) (the most dominant species), *Mesopolobus morys* (Walker) and *Stenomalina gracilis* (Walker) (Kevväi *et al.*, 2006; Veromann *et al.*, 2006a, b, c)

In addition to insect pests, the yield can be grately reduced by diseases of oilseed rape. The most serious diseases are blackleg [*Leptosphaeria maculans* (Desm.) Ces.], and stem rot [*Sclerotinia sclerotiorum* (Lib.) de Bary]. To date, dark spot disease [*Alternaria brassicae* (Berk.) Sacc.] is the most frequent disease found on winter oilseed rape fields in Estonia (see the Final report of MASTER – MAnagement STrategies for European Rape pests EU-QLK5-CT-2001-01447 http://www.rothamsted.bbsrc.ac.uk/pie/master/master.htm).

The aim of this study was to determine if and how mineral N-fertiliser influences: the number of pods per plant; the abundance of *M. aeneus* larvae in flowers and their paratism; the pod damage caused by *C. assimilis* and their larval parasitization rates; and on the occurrence of plant diseases in winter oilseed rape.

Material and methods

This study was conducted on an experimental field at Jõgeva Plant Breeding Institute, Estonia. The experiment was established with three replicates of seven different N-fertilizer levels: 0, 60, 80, 100, 120, 140 and 160 kg/ha of active N. Plot size was 10 m^2. of winter oilseed rape cv. *Silva* (seeding rate 6 kg/ha), drilled on 15 August 2007, following a fallow. Before sowing, fertilizer Kemira Power 5-10-25 S Fe B (300 kg/ha) was applied. No herbicides, insecticides or fungicides were applied in this experiment. The N-fertilizer ammonium nitrate (N 34.4%) was applied according to the experimental design (N 0-160 kg/ha) on 23 April 2008. The crop was harvested on 7 September 2008. The number of pods was counted at plant growth stage BBCH 80-81 (Lancashire *et al.*, 1991, Meier, 2001) on three plants per plot, to estimate the impact of fertilisation on yield. In addition, the nitrogen efficiency was calculated and the glucosinolate content and the yield were measured. Agronomic N-efficiency (Eq. 1) indicates effectiveness of fertilizer N-recovery as a result of N-uptake by the plant (Craswell & Godwind, 1984, Rathke *et al.*, 2006)

Equation 1.

$$\text{Agronomic N-efficiency} = \text{seed yield}_{\text{fertilized}} - \text{seed yield}_{\text{unfertilized}} \times \text{N supply}^{-1}$$

For estimations of oviposition activity and larval parasitization levels of *M. aeneus*, larvae were collected from oilseed rape flowers (at BBCH 67-68) from five randomly chosen plants on each plot. Larvae were counted, second instar larvae were dissected in the laboratory and the percentage parasitism calculated. The establishment of damage and parasitization parameters of *C. assimilis* was assessed at BBCH 80-81. Five pods from the main raceme and five from third side branch were collected from five randomly chosen plants per plot. The pods were incubated in emergence traps (Figure 1) in the laboratory. Four weeks later, emerged parasitoids and exit holes on pods were counted and the percentage larval parasitism and damaged pods were calculated.

Visual disease assessments were made at plant growth stage BBCH 80-81; five plants were randomly chosen from each plot. The abundance of *A. brassicae* lesions on leaves, stems and pods was assessed on scale from 0-6 (0: no disease; 1: 1-5%; 2: 5-10%; 3: 10-20%; 4: 20-30%; 5: 30-50%; 6: more than 50% of surface area covered with lesions), based on Conn *et al.*, 1990.

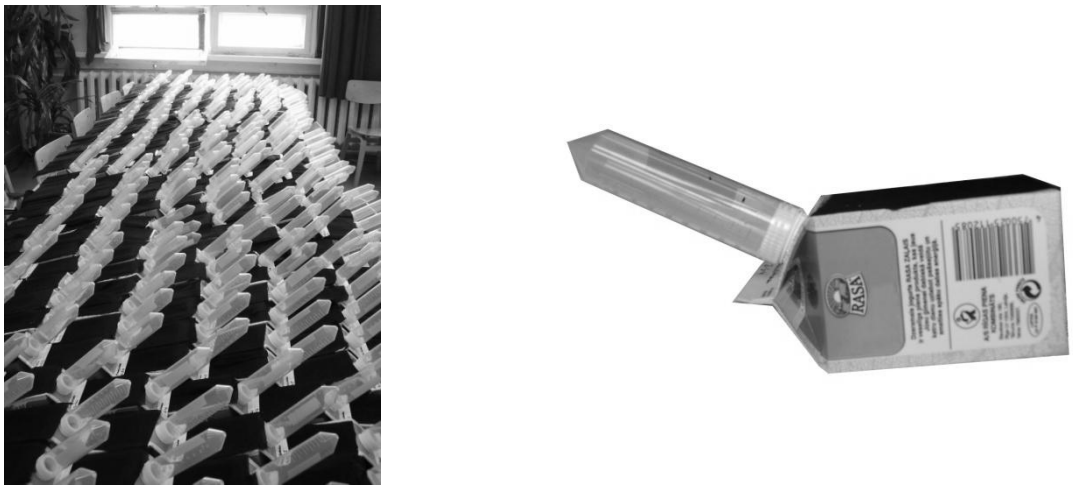

Figure 1. Emergence traps for rearing parasitoids of *Ceutorhynchus assimilis*.

The means of pest and parasitoid numbers were compared using the least significant difference test (LSD); the impact of fertilisation on disease abundance was found with one-way ANOVA Tukey test in Statistica 6 (StatSoft Inc. USA).

Results and discussion

Pods and yield

The number of pods per plant was significantly different between the fertilizer treatments ($F_{6, 56}$) = 5.52, $P < 0.0001$) and showed a strong increase in pod number (Figure 2) and yield (Figure 3) with increasing N. Plants in plots with no external N-supply (N 0) and 60 kg/ha N-supply (N 60) had significantly less pods than plants in plots with nitrogen 80, 100, 120,140 and 160 kg/ha ($P < 0.005$), whereas the differences among the high N rates were not statistically significant ($p > 0.05$). It has been shown before that variation in pod number of oilseed rape is essentially dependent on availability of N during growth and development. Since winter oilseed rape is a heavy N consumer, available N is the most limiting factor for growing this crop (Rathke *et al.*, 2006). Additional application of N in spring increased photosynthetically active leaf canopy, maintaining strong healthy plants and resulted in higher number of pods per plant. There was 2.7-fold difference in yield between the control plot (N 0) and N 160 plot (Figure 3). The significant increase in yield occurred in 80 kg/ha N-supply (N 80) compared with N 0 ($P < 0.025$). In addition, plants in plots N 80 and N 100 assimilated the most effectively external supplied nitrogen (Figure 4). N-efficient cultivars are those able to achieve seed yield above average under limited (Graham, 1984) as well as under high N-supply conditions (Sattelmacher *et al.*, 1994; Rathke *et al.*, 2006).

Content of glucosinolates in seeds fluctuated with the least in N 100 plots and the most in the control plots (Figure 5). Along with increasing rate of N-fertilizer, the yield of raw oil content decreased substantially (Figure 6). The highest oil content (48.3%) was recorded with unfertilized winter oilseed rape, while the lowest (42.2%) appeared at the highest N-supply. A decrease in oil content of seeds has been reported before with use of N-fertilizer (Barlog & Grzebisz, 2004; Rathke *et al.*, 2005, 2006).

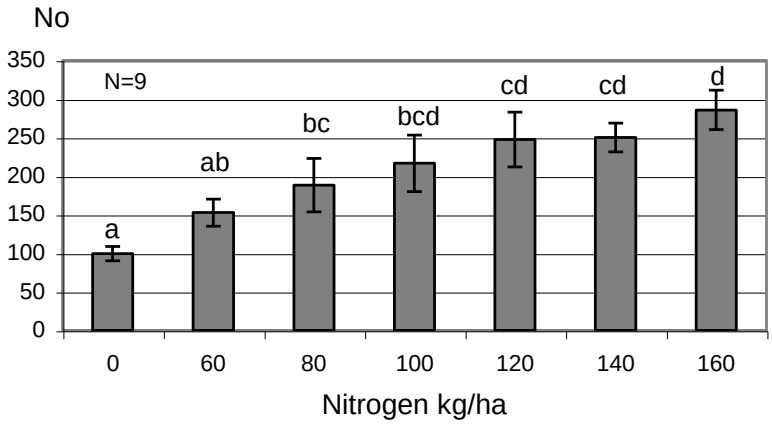

Figure 2. Mean number of pods (± SE) per winter oilseed rape plant at different N-fertilizer levels in Jõgeva County, Estonia, 2008 (letters indicate significant differences between treatments $P < 0.05$).

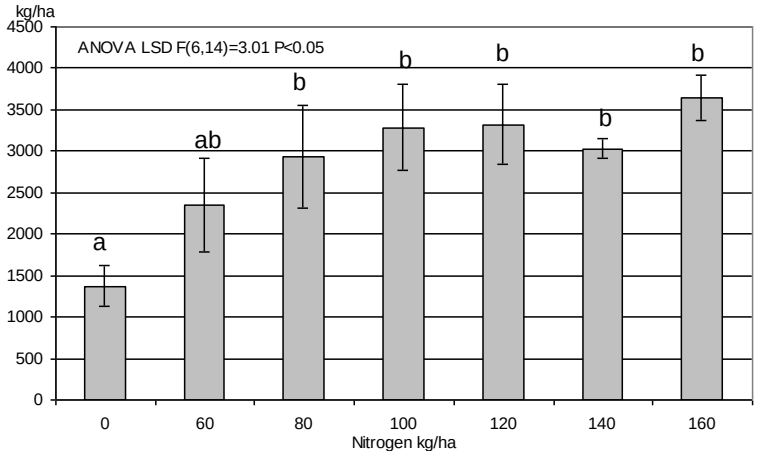

Figure 3. Mean yield (± SE) of winter oilseed rape at different N-fertilizer levels in Jõgeva County, Estonia, 2008 (letters indicate significant differences between treatments $P < 0.05$).

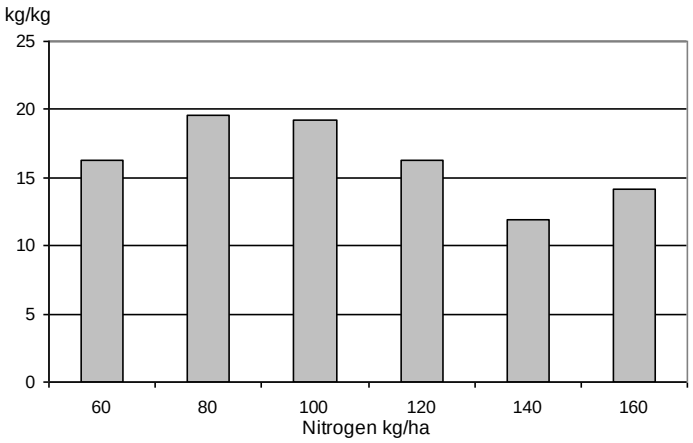

Figure 4. Agronomic N-efficiency of winter oilseed rape at different N-fertilizer levels in Jõgeva County, Estonia, 2008.

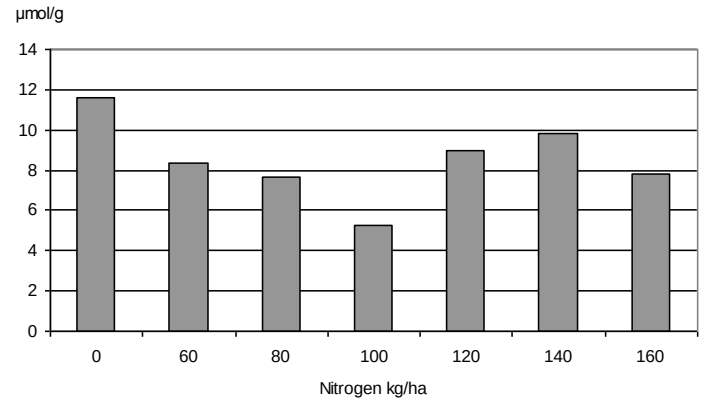

Figure 5. Mean content of glucosinolates in winter oilseed rape seeds at different N-fertilizer levels in Jõgeva County, Estonia, 2008.

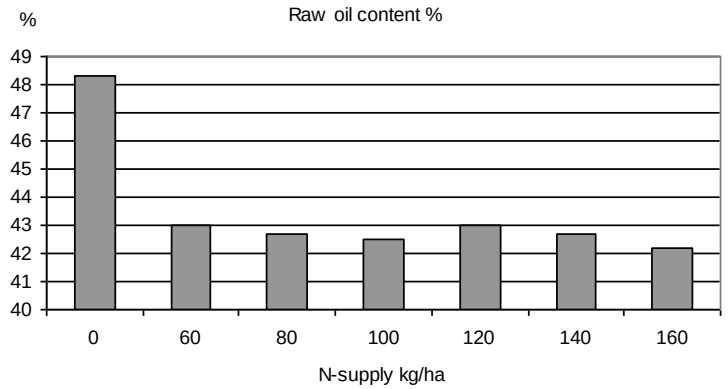

Figure 6. Mean content of raw oil in winter oilseed rape seeds at different N-fertilizer levels in Jõgeva County, Estonia, 2008.

Insect pests

The low oviposition activity of *M. aeneus* confirmed our previous findings that the phenological development of the crop was asynchronous with the phenology of *M. aeneus* (Veromann *et al.*, 2006b). The most attractive plants for *M. aeneus* were in plots N 80 and N 160 (Figure 7). The attractiveness of N 80 compared with plots with lower nitrogen amounts is explicable with the greater number of flowers (Figure 2). However, the number of flowers increased consistently with greater amounts of nitrogen, the ovipostition activity of beetles reduced notwithstanding the availability of greater number of suitable sites for egg laying. Nevertheless, the most preferred plants for *M. aeneus* were in plots with the highest nitrogen load (160 kg/ha) (Figure 7). Thus far it is assumed that nitrogen usage is unlikely to have any direct effect on the abundance of the oilseed rape insect pests (Walters *et al.*, 2003) and that only indirect influence on the searching efficiency of hymenopterous parasitoids through the plant architecture and inner microclimate of crop would be likely (Walters *et al.*, 2003). However, it is clear that plant structure and the greater number of flowers was not the cause of the decrease of oviposition activity from N 100 to N 140. One possible explanation might be the chemical response of plants to N fertilisation. The volatile chemicals such as glucosinolates, are important clues to host selection by cruciferous pests and also their parasitoids, aiding both finding and recognition/acceptance of the host plant (Bartlet, 1996; Alford, 2003). The content of glucosinolates in seeds (Figure 5) fluctuated compared to the

abundance of the *M. aeneus* larvae. Seeds of the most unattractive plants for *M. aeneus* (N 0, N 120, N 140) contained higher rates of glucosinolates. The mechanisms of differential preference of pests are based on certain visual and semiochemical stimuli (Cook *et al.*, 2007a, b). Therefore, it is probable that at certain nitrogen quantities plants could have had the synergy of chemical reactions and emitted certain semiochemical stimuli in different combinations and volumes which in combination with visual cues were attractive or repellent for adults of *M. aeneus*. To prove the impact of fertilization on the emission of semiochemicals, additional studies are needed.

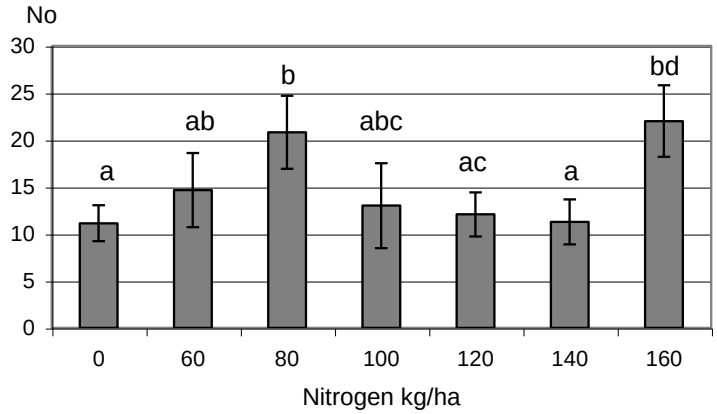

Figure 7. Mean number of *Meligethes aeneus* larvae (± SE) per winter oilseed rape plant at different N-fertilizer levels in Jõgeva County, Estonia, 2008 (letters indicate significant differences between treatments $P < 0.05$).

In Estonia, in accordance with our previous findings (Veromann *et al.*, 2006a, b, c), *C. assimilis* was more abundant in winter oilseed rape than *M. aeneus*. In this study, the level of *C. assimilis* pod infestation was low and below the threshold of economic losses (which is 26% (Free & Williams, 1978, Lerin, 1984, Buntin, 1999). Compared to *M. aeneus* damage, the pods attacked by *C. assimilis* were more evenly distributed. However, plants in plots N 140 had lower percent of damaged pod than all others (Figure 8).

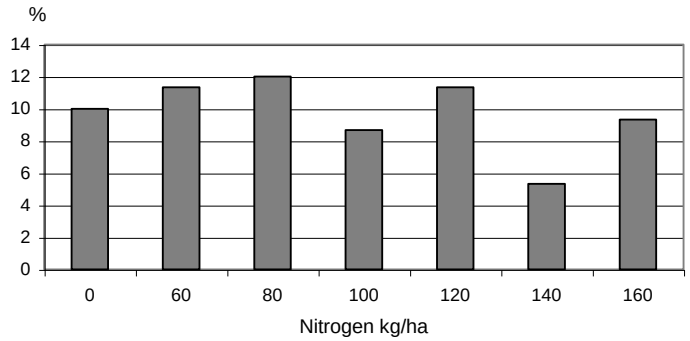

Figure 8. Mean percent of damaged pods caused by *Ceutorhynchus assimilis* per winter oilseed rape plant at different N-fertilizer levels in Jõgeva County, Estonia, 2008.

Parasitoids

The parasitization level of *M. aeneus* larvae was low in all plots and stayed below 10% even in the most infested plots. The success in host finding of *M. aeneus* parasitoids was the greatest in plots N 160 and the lowest in N 80 ($P < 0.05$) (Figure 9). Parasitoid number decreased with increasing host number, except for N 160, which was the peak for both of them. Therefore, comparing the behaviour of hosts and parasitoids we can assume that the attractiveness of plants with intermediate nitrogen fertilisation was converse to parasitoids compared to their insect hosts. In addition, the plants with higher glucosinolate content in the seeds (Figure 5) appeared to be more attractive for parasitoids and unattractive for their insect hosts than plants with lower glucosinolate levels. Thus, there was a slight tendency that these plants pushed *M. aeneus* away and pulled parasitoids into plots. The common parasitoids of *M. aenus* larvae were *Diospilus capito* (Nees) (the most abundant), *Tersilochus heterocerus* (Thomson) and *Phradis morionellus* (Holmgren) (Figure 10).

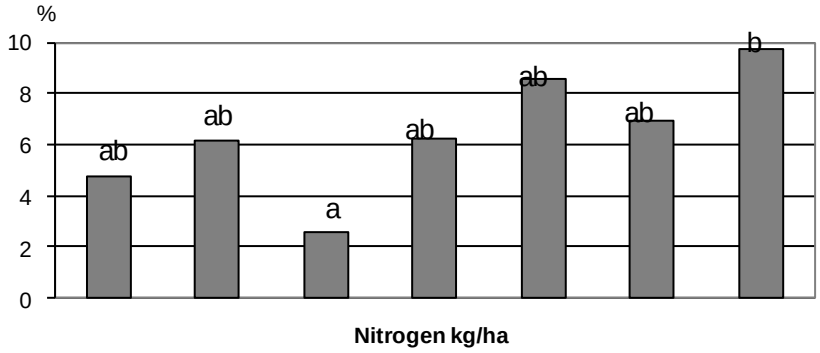

Figure 9. Mean parasitism rate of *M. aeneus* larvae at different N-fertilizer levels in winter oilseed rape in Jõgeva County, Estonia, 2008 (letters indicate significant differences between treatments $P < 0.05$).

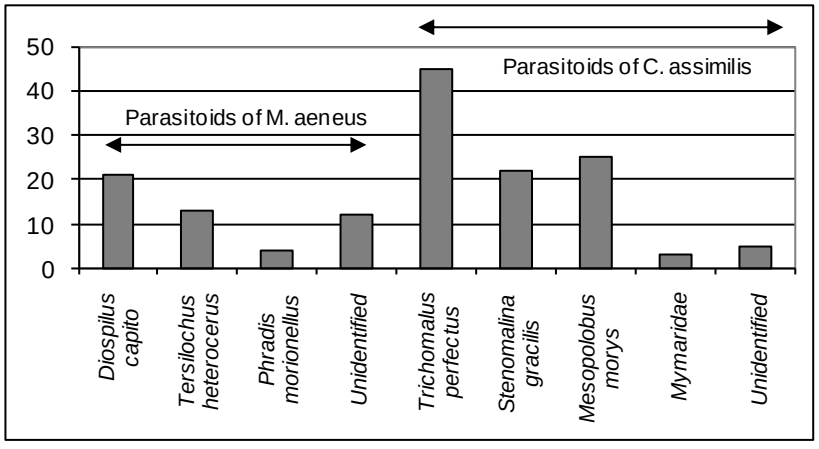

Figure 10. The species composition and the total number of parasitoids of *Meligethes aeneus* (dissected from larvae) and *Ceutorhynchus assimilis* (reared from pods) of winter oilseed rape, in Estonia, 2008.

The parasitization rate of *C. assimilis* was correlated to host abundance and therefore the impact of additional nitrogen was not detected. The level of parasitism was 100% in all plots except N100 and N160, where it was 92.3 and 92.9%, respectively (Figure 11). This remarkably high rate of parasitization shows that parasitoids of *C. assimilis* have built up a viable population and can efficiently control the population size of their host. Three important larval parasitoids emerged: *Trichomalus perfectus* (Walker) (the most dominant species), *Mesopolobus morys* (Walker) and *Stenomalina gracilis* (Walker).

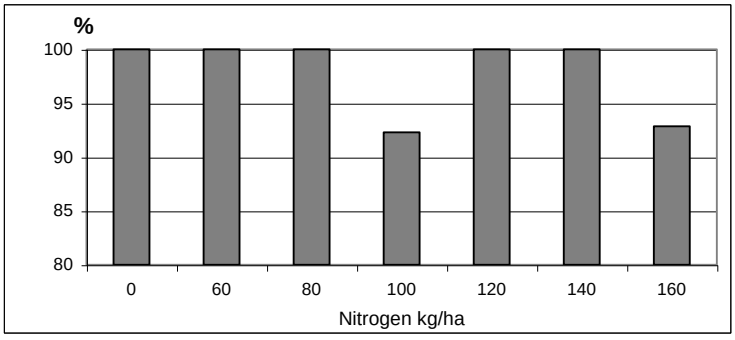

Figure 11. The parasitism rate of *Ceutorhynchus assimilis* per winter oilseed rape plant at different N-fertilizer levels in Jõgeva County, Estonia, 2008.

Diseases

Alternaria brassicae was the main disease problem found in this experiment. Dark lesions occurred on all plant parts, whereas the stems were as infected as the pods. The weather conditions during the growing season were humid and cool, which are very favourable for *A. brassicae* spread and disease development (Hong *et al.*, 1996). In addition to *Alternaria* blight, gray mould (*Botrytis cinerea* (de Bary) Whetzel.) was found on some of the plants. However, this disease was not frequent and was present only on a few pods of less than half of the plants evaluated. No other oilseed rape diseases were found during this study.

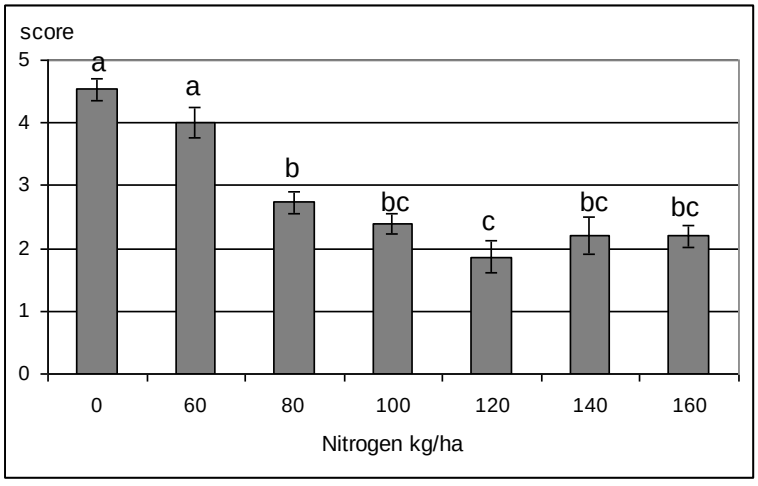

Figure 12. Mean disease score on scale 0-6 for *A. brassicae* at different N-fertilizer levels in winter oilseed rape in Jõgeva County, Estonia, 2008 (letters indicate significant differences between treatments $P < 0.05$).

The average disease score for *A. brassicae* per plant was significantly dependent on fertilisation ($F_{6, 98} = 22.02$, $P < 0.0001$). Plants with the highest damage were found in control plots (N 0) and in the case of minimum fertilisation (N 60). The plants with nitrogen levels between 80-160 kg/ha had significantly less disease lesions ($P < 0.001$), whereas the differences between them were not significant ($P > 0.05$) (Figure 12). Our results indicate that nitrogen fertilisation does not increase *Alternaria* spot disease occurrence on winter oilseed rape, as indicated in the case of many other diseases (Söchting & Verreet, 2004; Mert-Türk *et al.*, 2008). On the contrary, this experiment showed that nitrogen fertilisation helps the plant to cope better with the disease pressure, which can be explained by better growing conditions for plants and higher self-defence capacities.

Conclusions

The study showed a positive effect of N-fertilisation on pod number per plant and a negative effect on the occurrence of dark spot disease. Since there was no significant difference between treatments from N 80 to N 160, it could be recommended to use intermediate fertilizer levels, which would be more environmentally friendly. The oviposition activity of *M. aeneus* was lowest from N 80 to N 160 and in *C. assimilis* was the lowest in N 140. Therefore, the preferable quantity of nitrogen to suppress insect damage is 100-140 kg/ha. A parasitism rate of 100% *C. assimilis* larvae showed that in addition to optimised N-supply, parasitoids can contribute significantly to integrated pest control. Thus, this study supports the possibility that with carefully chosen management it is possible to achieve environmentally and economically beneficial reductions in the use of nitrogen and pesticides.

Acknowledgements

We thank Reelika Kevväi for field assistance and Jõgeva Plant Breeding Institute for crop husbandry. This study was supported by the Estonian Science Foundation SF0172655s04.

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Orobanche ramosa on winter oilseed rape in France: risks and perspectives of control

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Abstract: *Orobanche ramosa* (Broomrape) is an obligate parasitic plant affecting several crops such as oilseed rape, tobacco, hemp or melon. This parasite causes yield losses by competing against its host for nutrients and water. Its presence has been reported for a long time in France, but it has caused severe damage in oilseed rape crops since about 1990, especially in the region Poitou-Charentes. The area affected by *O. ramosa* continues to expand, and no effective methods are available to reduce the field infestations and damage caused by *O. ramosa*. Several factors make the control of *O. ramosa* difficult, notably the high number and the viability of seeds produced by broomrape and the potential of broomrape seed germination in a large range of environments. In recent years, different methods to control broomrapes were investigated, either to reduce broomrape seed banks in infested fields, or to limit the impact of the parasite on crop yield.

The best way to reduce seed bank in soil is the induction of suicide germination, combined with the limitation of seed production by plants. The germination of *O. ramosa* seeds occurs only in response to stimulants present in exudates from host roots. A set of species was screened in order to identify species that induce the germination of *Orobanche* and that enable (trap-species) or not (false-hosts) the parasite to reach ripeness. The introduction (i) during the intercropping period of trap-species, that have to be destroyed to stop the development of the parasitic plant, or (ii) of false hosts in the rotation, provides promise to reduce the soil seed bank. Simultaneously, weeds are also tested for their susceptibility to *Orobanche* in order to advise farmers on weeding measures to limit the number of potential hosts in field.

Different cultural practices were also tested to limit the infestation of oilseed rape by *Orobanche* and the most promising method was the use of plant genetic resistance. Genetic variability of oilseed rape cultivars was investigated in order to find resistance mechanisms. Studies indicated that only partial resistance was detected. However, as the partial resistances identified seem to correspond to different mechanisms, it should be possible to accumulate them to create cultivars with increased resistance.

Key words: *Orobranche ramosa*, France, control, cultural practices, resistance

Assessing slug risk and slug control in oilseed rape

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Abstract: Slugs are among the most important pests of oilseed rape. In order to predict slug damage and the need for applying slug pellets in oilseed rape, trapping of slugs was conducted in stubble, prior to plant emergence (using upturned terracotta flowerpot saucers, 25 cm diameter, with 20 ml of chicken layers` mash placed in a small heap on the soil). The number of slugs counted in traps varied from 1 to 4; a sufficient abundance to justify the application of slug pellets. A field experiment was conducted in an oilseed rape crop from 20th September to 9th October 2007, from plant emergence to the four-true-leaf stage. In the field experiment three compounds: metaldehyde, methiocarb and Fe (III) pyrophosphate were tested for their ability to reduce the damage caused by *Arion* sp. Férussac 1819 (Gastropoda: Pulmonata: Arionidae). All three treatments of molluscicide protected against severe crop loss, with significant differences among methiocarb and other treatments in the last week of the assessment' this could be explained by the wet weather. However, harmful the effect of methiocarb to carabid beetle populations and earthworms was also evident.

Key words: slug, control, oilseed rape, Croatia

The effect of seed treatment on the growth and development of oilseed rape

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Abstract: The development and growth of oilseed rape depends on the choice of variety, fertilization, soil type, climatic conditions, and pest and disease infestation. In Croatia the oilseed rape seed sown is generally treated with the preparation Chinook FS 200, which enables the protection of this crop from autumn pests in the early development stages (BBCH 0-14). The aim of this investigation was to determine the effect of thiametoxam (Cruiser OSR FS) and imadicloprid (Chinook FS 200) on the pest incidence, growth and development of oilseed rape crops. The research was carried out during the vegetation period of 2007/2008 at two sites: Koprivnički Bregi and Popovača, Croatia. At Koprivnički Bregi, two visual inspections of the plants were made (November 13, 2007 and March 1, 2008) on the variety Mohican. Ten plants in six replicate crops were inspected. At Popovača, only one visual inspection was made, on March 4, 2008, with the variety Oase. Ten plants in three replicates were examined. The impact of the test active ingredients on the pests was low (pests infestation was very low). The impact on the growth and development of the oilseed rape plants was determined by measuring the length of the root, the weight of the plant, the number of leaves and the number of plants per square meter. Through a statistical analysis of the data obtained it was found that the number of leaves on the plants whose seed had been treated with thiametoxam was significantly greater than that on the plants treated with imidacloprid. The results from the first year of this experiment suggest that the research should be continued and expanded to determine the impact of thiametoxam on the growth and development of different oilseed rape hybrids.

Key words: oilseed rape, seed treatment, Croatia, thiametoxam, imadichloprid

Entomology papers

The potential of the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuillemin and *Metarhizium anisopliae* (Metschinkoff) Sorokin to control *Helicoverpa armigera* (Hübner) on sunflower

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Abstract: The potential of two isolates of the entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuillemin (WG02; WG03) and *Metarhizium anisopliae* (Metschinkoff) Sorokin (WG04; WG05) was investigated under laboratory conditions against 2nd instar larvae and the pupae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on sunflower. The fungi were used alone or in all the possible combinations (WG02; WG03; WG04; WG05; WG02 + WG04; WG02 + WG05; WG03 + WG04; WG03 + WG05 and WG02 + WG03 + WG04 + WG05) at three dose rates: 10^5, 10^7 and 10^9 conidia ml^{-1} of each treatment. The mortality of 2nd instar larvae of *H. armigera* was affected at all dose rates of the fungal isolates tested and also the emergence of the pupae was reduced. The results of the present study clearly suggest that the blending of the four isolates has a greater bio-efficacy and potential to control both the larvae and the pupae of *H. armigera*.

Key words: *Beauveria bassiana*, *Metarhizium anisopliae*, *Helicoverpa armigera*, sunflower, Pakistan

The effect of fertilizers on the pests in oilseed rape

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Abstract: The use of petroleum products in Croatia and in the world at large is on the rise. It is expected that the consumption of motor fuels will rise because of the rapid development of transportation, while the consumption of fuel for heating will decrease. On the other hand, the production of crude oil will fall from 1.37 million tons in 2000 to 0.6 million tons in 2010, while in 2030 a production of no more than 0.4 million tons of crude oil is forecast. An alternative to mineral fuels is the production of biodiesel from various plant oils, as well as from beef suet and used cooking oils (from households and restaurants). The most important raw materials for the production of biodiesel are oilseed rape (82.82% of the total) and sunflower (12.50%). The production of biodiesel in the world is constantly rising, and the areas of land devoted to oilseed rape and other crops for the production of biodiesel are expanding, which results in an increased need to protect these crops from diseases, pests and weeds. In order to achieve high yields, fertilizer application is of great importance, and yet if it is excessive, it can have a negative effect with respect to the incidence of pests. Therefore the aim of this research was to determine the effect of various quantities of fertilizer on the abundance of the weevils *Ceutorrhynchus napi* and *Ceutorrhynchus pallidactylus* (Coleoptera: Curculionidae) on oilseed rape. The experiment was set up with the Triangle variety of oilseed rape on the Vinokovščak site (Varaždin, Croatia) during 2008. The investigation was conducted on a 1 hectare field, divided into three plots. Fertilizer (KAN) was applied to the plots at three different quantities: 150, 200 and 250 kg/ha. During the vegetation period, two visual inspections of the oilseed rape plants were made: on April 20 (BBCH 62-64) and May 31 (BBCH 77-79). Ten plants in four replicates were taken from each experimental field. The plants were dissected and the number of perforations, lengths of feeding galleries and number of *C. napi* and *C. pallidactylus* larvae were recorded. Statistical analyses of the results showed that there were significant correlations between pest attack and the differing quantities of fertilizer; larval abundance increased with higher amounts of fertilizer. The data obtained show the need to analyze the total amount of nitrogen in the soil and to apply fertilizers at a rate that appropriately reflect its actual nitrogen content to ensure that the needs of the crop (oilseed rape in this case) for nitrogen are met but not exceeded.

Key words: oilseed rape, interaction pests – fertilizer, *Ceutorrhynchus* sp., Croatia

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Effect of nitrogen fertilization, cultivar and species on incidence of two major pests of winter oilseed rape (*Brassica napus* L.): the pollen beetle (*Meligethes aeneus* F.) and the stem weevil (*Ceutorhynchus napi* Gyl.)

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Abstract: Pollen beetle and stem weevil are among the most important insect pests in winter oilseed rape crops (WOSR). Moreover recent monitoring programs have shown the important development of metabolic resistance to pyrethroïds resulting in inefficient insecticide treatments. A better comprehension of the relationships between crop management and pest damage has been investigated in order to adapt new control strategies. To understand the effects of crop management, we measured population dynamics and damage caused by both pollen beetles and stem weevils on a split plot testing three nitrogen supply levels and two cultivars as main factors. Plant species effects were investigated in an experimental trial of winter oilseed rape with turnip rape borders. A cultivar with high isothiocyanate concentrations was more attractive than a cultivar with low isothiocyanate concentrations. Crop attractiveness is function of nitrogen supply and its effect on different crop variables. Indeed, stem weevil selects its host plants at stem elongation and is affected by crop height, whereas pollen beetle is sensible to dry weight. Furthermore a major role of growth stage development on host selection was found: the more advanced stages were the more colonized. These effects have not been previously reported at field level. Our results confirmed a high attractiveness of the turnip rape due to growth stage. No effect of nitrogen supply and cultivar was reported on the number of damaged buds. However, clear effects of nitrogen on stem weevil damage were recorded. Nitrogen fertilization interacts with pollen beetle damage by compensation mechanisms principally acting on seed weight. Compensation capacities are determined by time and rates of nitrogen applications and by pest population dynamics. These results bring new challenges for crop management, particularly for organic crops, by trap crop strategies and adapted nitrogen applications.

Key words: oilseed rape, *Meligethes aeneus*, *Ceutorhynchus napi*, nitrogen supply, isothiocyanates, cultivar, trap crop

Introduction

The pollen beetle (*Meligethes aeneus* Fabricus) (Coleoptera: Nitidulidae) and the stem weevil (*Ceutorhynchus napi* Gyl.) are two major pests of winter oilseed rape (*Brassica napus* L.) (Brassicaceae) in Europe (Alford *et al.*, 2003). The pollen beetle feeds on pollen from flowers and buds, and females lay their eggs in buds suitable for oviposition (2-3 mm long) (Nilsson, 1994). Rape stem weevil females lay their eggs in the stem, deforming the stem considerably, and thereby damaging the crop. Serious yield losses, over 70%, can result from pollen beetle attacks due to bud abortion (Nilsson, 1987; Ekbom & Borg, 1996). The main method for control of these pests is the intensive use of insecticides, but the emergence of metabolic resistance to pyrethroids is now widespread. New strategies for insect pest management appear to be a key issue for WOSR cropping. In the literature, host quality is considered as an important factor of host selection mechanisms by insect pests. Several authors have reported that pollen beetle and stem weevil locate their host plants using visual and olfactory cues and

particularly isothiocyanates (ITC) odours (catabolites of the glucosinolates) (Evans & Allen-Williams, 1998; Smart & Blight, 2000; Cook *et al.*, 2006). It has also been reported that the glucosinolate content of the plant depends on different agronomic factors such as sowing date, plant density, fungicide treatments or nitrogen supply (Milford & Evans, 1991). The major role of nitrogen supply on glucosinolate production on the aerial parts of the plant was reported by Markus *et al.* (1996). Moreover, different studies have focused on the impact of trap crop strategies based on turnip rape or different cultivars (Cook *et al.*, 2003; Cook *et al.*, 2006). They demonstrated that oilseed rape (particularly the low ITC cultivars) were systematicaly less colonized by pollen beetle than turnip rape plants.

Studying the interactions between nitrogen supply and cultivar or species appear to be the cornerstone of the development of integrated pest management at the field scale. Indeed, nitrogen fertilization, cultivar and species may play a significant role in attractiveness of the crop through "push-pull" actions. Furthermore nitrogen application and cultivar may have an important effect on compensation mechanisms and on pest damage. The objectives of this study is to understand the effects of some crop management elements on crop attractiveness and pest damage in order to develop new control strategies.

Material and methods

Experimental design

In 2007, a split plot testing three nitrogen fertilization levels (differing by time and rates of application; N0: 80 kg/ha in one early application, N1: 160 kg/ha in two early applications and N2: 160 kg/ha in two late applications), two cultivars (*A* and *B*) and two pesticides treatments (total protection and no protection) as main factors, was conducted at Grignon, France. The two cultivars differed by their glucosinolate contents of aerial parts. In 2006, an experimental trial with a 6 m turnip rape (*Brassica rapa*) border was conducted in Versailles, France.

Crop variables and insect sampling

Crop condition was described by different variables, such as nitrogen accumulation, phenological growth stage, height, weight, stem diameter and isothiocyanates (ITC) content. Isothiocyanate concentrations were only measured at one stage, and were done using high performance liquid chromatography methods (HPLC). Pollen beetle populations were counted weekly on 30 plants for each modality. The number of stem weevil punctures were counted on the main raceme at growth stage 6.3 and were used to estimate stem weevil incidence. Dry biomass (after 48 h of drying at 80°C) and total nitrogen content of green aerial parts were determined for each microplot sample. We calculated the nitrogen nutrition index (NNI) as the ratio between the nitrogen content of the aerial parts of the plant and critical nitrogen content, using the reference values of critical N content for oilseed rape. The numbers of branches, flowers, fertile pods and podless stalks per plant were counted on 10 randomly chosen plants per microplot at the pod-filling stage. Damage resulting from pollen beetle were evaluated by comparisons with the control (treatment with insecticides was assumed to give complete control).

Data analysis

The effects of the three nitrogen levels and the two cultivars on abundance of, and damage by adult pollen beetle and stem weevils were assessed using a two-way ANOVA. Effect of turnip rape borders was analysed by one-way ANOVA. Response variables were tested for normality using Shapiro-Wilk W-statistic and were transformed to meet criteria for statistical analysis. For example proportions, such as the proportion of plants attacked by rape stem weevil or the proportion of podless stalks resulting from pollen beetle attacks were analysed after an arcsin √ transformation to stabilise variance between groups. All statistical analysis were performed using SAS software (SAS Version 8.2, SAS Institute Inc., Cary, NC, USA).

Results and discussion

First of all, no significant differences on the total number of adult beetles were observed between the two cultivars (Figure 1a). However, we observed significant differences between cultivars *A* and *B* on the number of pollen beetles per plant at each stage. *A* was more attractive until growth stage (GS) 3.5, and *B* was more attractive from GS 3.5 to GS 4.5 (data not shown).

Secondly, we found a relationship between nitrogen supply level and crop attractiveness. In fact, pollen beetle were found in large numbers on plants of the nitrogen modalities N0 and N1 (Figure 1a). Plants of the N2 modality were nitrogen deficient because of dry climatic conditions during N2 level application. Therefore N2 plants developped fewer buds and weaker buds not suitable for oviposition. An important effect of phenological stage on host plant selection was also recorded. The more advanced stage was systematicly more colonized by adults pollen beetle until flowering (GS 4.5).

Plant species seems to have a great importance because more pollen beetle was found on turnip rape than on WOSR (Figure 1b). An effect of the phenological stage on the number of pollen beetles was also found between the two different plant species and is reported as one of the important factor responsible for the greater attractiveness of turnip rape. As the glucosinolate content of the turnip rape aerial parts were not measured, no conclusions on which factor is reponsible for this higher attractiveness can be made.

We also found an effect of cultivar and nitrogen supply on stem weevil attacks and damage (Figures 2a and 2b). Cultivar *A* was more attractive than *B*, and plants of the modalities N0 and N1 had more stem weevil punctures. This result comes from the relationship between the peak of stem weevil flight and period of nitrogen application. Indeed, N2 plants were nitrogen deficient and were smaller plants than plants of the the two others modalities. As the stem weevil selects hosts on the basis of stem elongation, plants from treatment N2 showed less stem weevil punctures and less damage (Figures 2a and b).

Glucosinolate content of aerial parts at GS 4.5 differed between the two crop cultivars and are related to pollen beetle infestation levels. This result has never before been reported from field trials. Glucosinolate content was not related to nitrogen fertilization level at this stage. In order to explain if differences of infestation are directly related to glucosinolate content of aerial parts, more analyses are necessary at each growth stage of the crop.

No effects of nitrogen supply or cultivar were found on the percentage of buds damaged by pollen beetle. But we found an interaction between nitrogen fertilization and pollen beetle damage. Indeed, compensation mechanisms have been found principally occurring on seed weight.

1a

		Mean number of pollen beetle / plant	*F value*	Prob.
cultivar	A	4.48	2.76	*
	B	5.06		
Nitrogen supply	N0	4.9	12.69	***
	N1	5.7		
	N2	3.6		
Bloc				NS
N * cultivar				NS

1b

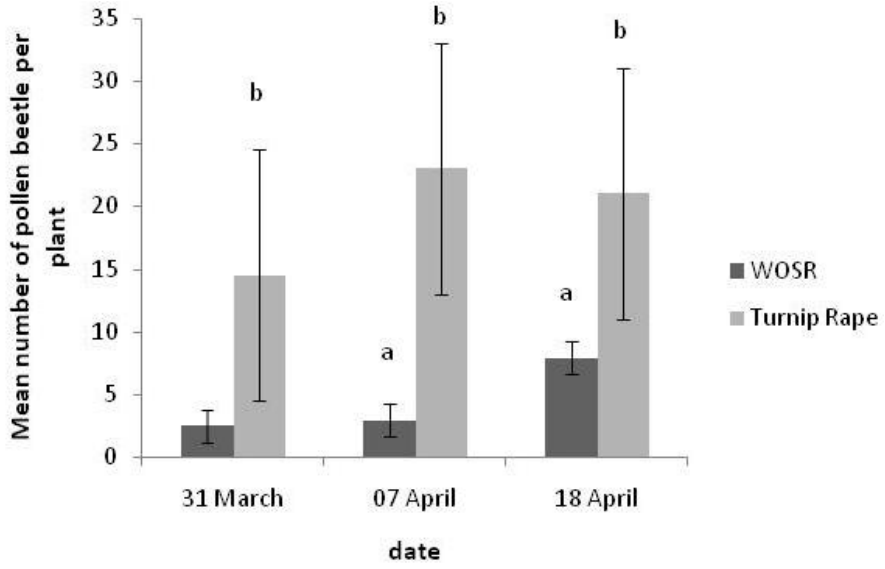
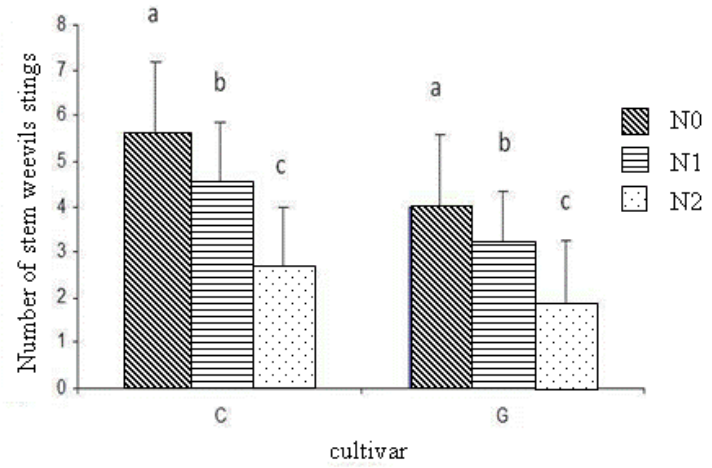


Figure 1: 1a**.** Effects of the different factors on mean number of pollen beetle for all dates (5 different dates), their F value and their means.***: P < 0.001; **: P < 0.05; * P < 0.1. 1b. Effects of turnip rape borders on the mean number of pollen beetle per plant at different stage of the crop.

The effect of nitrogen supply (time and rate) on the attractiveness and the capacity of plants to cope with a serious attack of pollen beetle and stem weevil has never before been reported. We found an important interaction between attack dynamic of pollen beetle and nitrogen supply. We have also shown an important effect of nitrogen supply on host quality and its impact on stem weevil damage which is dependent on the coincidence with the peak in flight activity of the stem weevil. Moreover, the effects of glucosinolates observed in the field experiment at one stage is a very interesting result and needs further experiments in order to understand how variation of glucosinolate content in the aerial parts of the plant and the impact of nitrogen supply on their concentrations are related to pest incidence. Even though these results have to be further explored they highlight some tactics that can be used to build integrated pest management strategies in order to reduce reliance on agrochemical treatments.

2a



2b

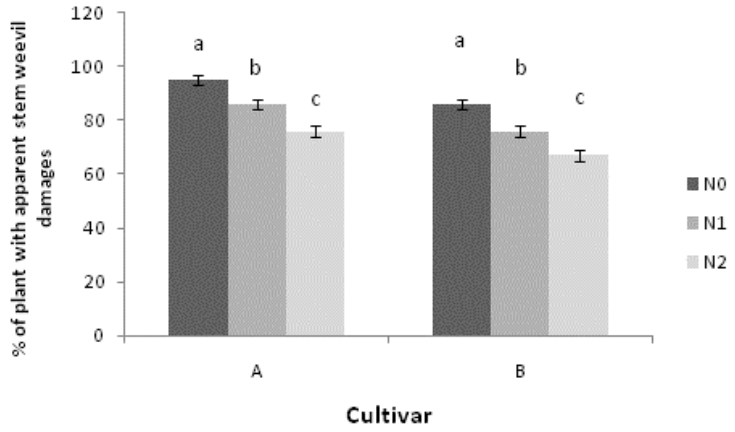


Figure 2: 2a. Mean number of stem weevil punctures on the main raceme for the two cultivars and the 3 N modalities (± SE). 2b**.** Percentage of plants with apparent stem weevil damage for the two cultivars and the 3 N modalities (± SE)

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Phenologies and diel periodicities of within-crop flight by pests and parasitoids in winter oilseed rape in the UK

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Abstract: Parasitoids are important natural enemies of insect pests of oilseed rape (*Brassica napus*) in Europe yet they are vulnerable to effects of insecticides. Temporal targeting of insecticide against the cabbage seed weevil (*Ceutorhynchus obstrictus*, syn. *C. assimilis*) reduces harm to its parasitoids and so benefits conservation biological control. The objective of this study was to establish whether the same principle could be used to protect parasitoids of other pests when insecticides are applied during bud or flowering stages for control of pollen beetle (*Meligethes aeneus*) or cabbage seed weevil. Yellow water traps in a crop of winter oilseed rape were used to record the phenology of these pests and of three species of tersilochine parasitoids, *Phradis interstitialis* and *Tersilochus heterocerus* (both larval parasitoids of pollen beetle) and *Tersilochus obscurator* (a larval parasitoid of cabbage stem weevil, *Ceutorhynchus pallidactylus*). The diel within-crop flight periodicities of the pollen beetle and two tersilochine species were established using Malaise traps with automated time-sorting heads and were analysed in relation to meteorological data. None of the parasitoids were active in the crop at bud stage and they would therefore not be at risk from insecticides applied at this recommended time for pollen beetle control. However, all three species were active in the crop at mid-flowering and therefore potentially at risk from insecticides applied for seed weevil control. Peak flight activity of the pollen beetle, *T. obscurator* and *P. interstitialis* was around midday and the pollen beetle and *T. obscurator* showed marked diel periodicity. Flight activity was positively correlated with solar energy and average air temperature and weakly negatively correlated with wind speed. Few insects were caught before 10.00 h. The difficulty of defining days when insecticide applications do not risk significant injury to parasitoids is discussed. It is suggested that parasitoids present in the crop might be less at risk from selective insecticides if applied a times of day when the insects are not flying and that this is worthy of further investigation.

Key words: pests, parasitoids, phenology, diel periodicity, flight, oilseed rape, insecticide, conservation biocontrol, temporal targeting, *Ceutorhynchus obstrictus*, *Ceutorhynchus assimilis*, *Meligethes aeneus*

Introduction

Parasitoids are of key importance in suppressing pests of winter oilseed rape throughout Europe and parasitism of 20-50% of pest larvae is common in the absence of insecticide use (Alford, 2003; Ulber *et al.*, 2006). However, adult parasitoids are vulnerable to insecticides used on the crop (Murchie *et al.*, 1997) and so chemical control risks becoming a self-perpetuating strategy as it alleviates pressure on pest populations from biocontrol agents. In turn, over-use of insecticides risks the development of insecticide resistance (Hanson, 2003).

In the UK, temporal targeting of insecticide against the cabbage seed weevil (*Ceutorhynchus obstrictus*, syn. *C. assimilis*) has been shown to reduce harm to its key parasitoid, *Trichomalus perfectus*, and so benefits conservation biological control (Murchie *et*

al., 1997). Using flight traps, it was shown that there was a time lag between the immigration flights of the weevil and *T. perfectus*, the weevil arriving in winter rape about two weeks earlier than its parasitoid. This provides an opportunity to apply insecticide to control the weevil before its parasitoid is present. However, in the UK, winter rape may be invaded during spring by a succession of four major pests: the pollen beetle (*Meligethes aeneus*), the cabbage seed weevil, the cabbage stem weevil (*Ceutorhynchus pallidactylus*) and the brassica pod midge (*Dasineura brassicae*), each having its own specific key species of hymenopterous parasitoids. It is not known whether the temporal targeting of insecticides against these pests could be used to protect their parasitoids or whether there are any predictable periods in spring when key parasitoids are not at risk in the crop.

Evidence for a temporal succession in the activity of parasitoids of rape pests already exists. Most overwinter in the soil beneath the crop, emerging in spring when they migrate to the current year's crop. In studies from 1998-2000 at Rothamsted on the spatio-temporal relationships between pests and parasitoids in winter rape (Ferguson *et al.*, 2003; Ferguson *et al.*, 2004; Ferguson *et al.*, 2006), the spring emergence of key parasitoids of pollen beetle, cabbage stem weevil and brassica pod midge, from the soil beneath the previous year's rape crop was recorded using emergence traps. However, this dataset did not allow spray windows to be determined as it did not establish the duration of activity of the parasitoids within the current year's crop, nor did it provide information about parasitoids that do not over-winter in the soil. Data from flight traps was therefore needed to determine the periods of parasitoid flight activity within winter rape in relation to the timing of insecticide application.

The risk posed by the application of an insecticide to a parasitoid in a crop is determined not only by the degree of toxicological selectivity of the insecticide and the presence/absence of the parasitoid in the crop but also by the behaviour of the parasitoid. Insects can acquire toxic doses of insecticide through direct contact with spray droplets during application, through contact with insecticide residues on plant surfaces or through ingestion of plant material with surface residues or systemically-absorbed insecticide. Many adult parasitoids make less intimate contact with plants than do their herbivorous hosts and they probably ingest less plant material, their main foods being floral or extra-floral nectar, honeydew and sometimes host haemolymph (Jervis, 1998). Therefore, contact with insecticide spray droplets during application may be a more important route for insecticide uptake for parasitoids than for their hosts. If selective insecticides were applied at times of day when parasitoids, though present, are not flying, direct contact of parasitoids with insecticide spray and their resultant mortality might be reduced. The diel flight periodicity of insect pests, particularly aphids and Lepidoptera, and the influence of light, temperature and wind, have been the subject of a number of studies in the last 50 years (e.g. Taylor, 1963; Lewis & Taylor, 1964; Walters & Dixon, 1984; Campbell & Muir, 2005). *Meligethes* sp. are reported to be day-flying (Lewis & Taylor, 1964) but the diel flight periodicities of other pests of oilseed rape and their parasitoids have not been determined.

The objective of this paper was to determine the seasonal and diel flight phenologies of key parasitoid species of oilseed rape pests at risk from insecticides applied in spring to control pollen beetle and cabbage seed weevil in winter rape in the UK, and to determine whether there is further potential for temporal targeting of insecticides to conserve parasitoids.

Material and methods

All sampling was conducted on Rothamsted Farm in the south-east of England in 2005 in a 300 m^2 plot of winter rape. From mid-February to mid-June, four yellow water traps (250 x 300 mm) at crop canopy height were used to record the phenology of the pollen beetle and the cabbage seed weevil and of three species of tersilochine parasitoids, *Phradis interstitialis* and *Tersilochus heterocerus* (larval parasitoids of pollen beetle) and *Tersilochus obscurator* (a larval parasitoid of cabbage stem weevil) and to search for 'spray windows' when the parasitoids would not be at risk from insecticides. Insect samples were collected three times a week and the growth stage of the crop was recorded on each sampling occasion.

At mid-flowering, the crop growth stage recommended for application of a pyrethroid insecticide to control seed weevil, the diel periodicities of within-crop flight by *T. obscurator*, *P. interstitialis* and pollen beetles, were studied in the same plot of winter rape using four Malaise traps modified to collect 12 two-hour samples every 24 h (Murchie *et al.*, 2001). The traps were run for five days from 09.00 h GMT on 28 April. Insect counts from the four Malaise traps were summed for each 2 h sampling period. The results were analysed in relation to meteorological data obtained from a site 1.6 km from the plot. Wind speed was measured at a height of 10 m, solar energy, temperature and relative humidity (RH) were recorded at 1.25 m above grass. The data were collated and evidence for diel periodicity of flight and the influence of meteorological factors was sought.

Results

Seasonal flight phenology of pests and parasitoids

Yellow water traps caught large numbers of pollen beetle and seed weevil and large numbers of pollen beetle parasitoids (*P. interstitialis* and *T. heterocerus*) but fewer *T. obscurator* (Table 1). Pollen beetle immigration began during 18-21 March at green bud stage (Figure 1). Between then and 1 April (the end of bud stage) 3214 pollen beetles were caught, representing 22% of their entire catch, but only one of their parasitoids was caught. All other tersilochine parasitoids immigrated during flowering, *T. heterocerus* rather later than *P. interstitialis* and *T. obscurator*, and all three species were present at mid-flowering when the cabbage seed weevil immigrated (Figure 1).

Table 1. Total numbers of insects trapped

Trap type	Trapping period	pollen beetle	seed weevil	*P. interstitialis*	*T. heterocerus*	*T. obscurator*
Water	18 Feb. - 17 June	14935	1370	604	7207	157
Malaise	28 April - 3 May	135	-	61	30	902

Diel periodicity of insect flight

Malaise traps caught sufficient pollen beetles, *P. interstitialis* and *T. obscurator* for analysis (Table 1). However, they caught few *T. heterocerus* and no cabbage seed weevil, even though water traps caught 357 and 64, respectively, during the five days from 27 April to 2 May.

Almost all *T. obscurator* and pollen beetle were caught in trapping periods during daylight hours (07.00-19.00 h), and, in each case, the numbers caught were distributed in a single broad peak with a maximum at mid-day (Figures 2 a & b). Autocorrelation analysis indicated strong 24 h periodicity in the activity of these insects, significant peaks and troughs in the autocorrelation function occurring regularly at 12 h intervals (Figures 2 d & e).

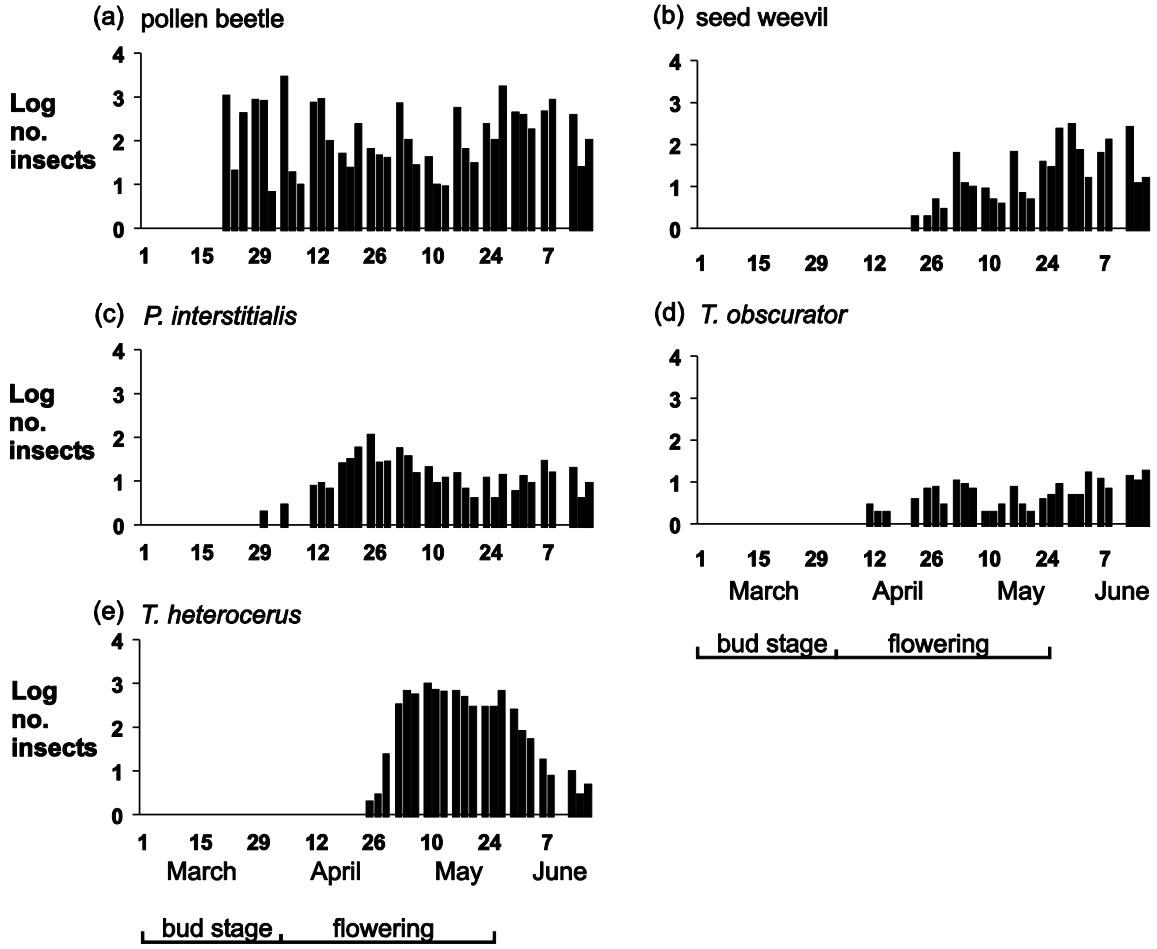

Figure 1. Phenology of insect catches in yellow water traps.

Phradis interstitialis also appeared to be day-active (Figure 2 c), but there was little evidence of periodicity in its autocorrelation function (Figure 2 f), probably because numbers caught were small and variable; even in daylight hours, fewer than half of the catches contained any of this species.

Influence of meteorological variables on insect flight

Average air temperature within each 2 h Malaise-trapping period ranged from 8.4 to 21.8 °C and showed diel periodicity (Figure 3), responding to solar energy with a 2 h time-lag (Table 2). As expected, average RH varied with inverse relation to temperature and also correlated

with solar energy, again with a 2 h time-lag (Figure 3; Table 2), but not with wind speed. Wind speeds varied from 2.8 to 12.0 m/sec, averaging 5.5 m/sec; this is equivalent to 2.1, 9.0 and 4.1 m/sec, respectively, at 1.25 m height and the speed experienced by the insects within the crop canopy would have been even less. Although it showed only weak evidence of diel periodicity, wind was usually stronger during the day than at night (Figure 3). There was no rain.

As the insects were day-active, only day-time trap catches were included in the analysis of the influence of meteorological variables in order to exclude the effect of the absence of light. Numbers of *T. obscurator* and pollen beetle in Malaise traps were positively correlated with both solar energy and temperature (Table 2). Numbers caught correlated more strongly with solar energy recorded in the previous 2 h trapping period (i.e. with a 2 h time-lag) than with solar energy at the time of trapping, whereas their correlation with air temperature was strongest without any lag (Table 2). By contrast, numbers of *P. interstitialis* correlated with solar energy without any lag and only weakly with other meteorological variables (Table 2). Plots of insect catches against air temperature suggest a flight temperature threshold of *c.* 14 °C for each of these insects (Figures 2 g-i). Catches of all three insects showed weak negative correlation with wind speed (Table 2).

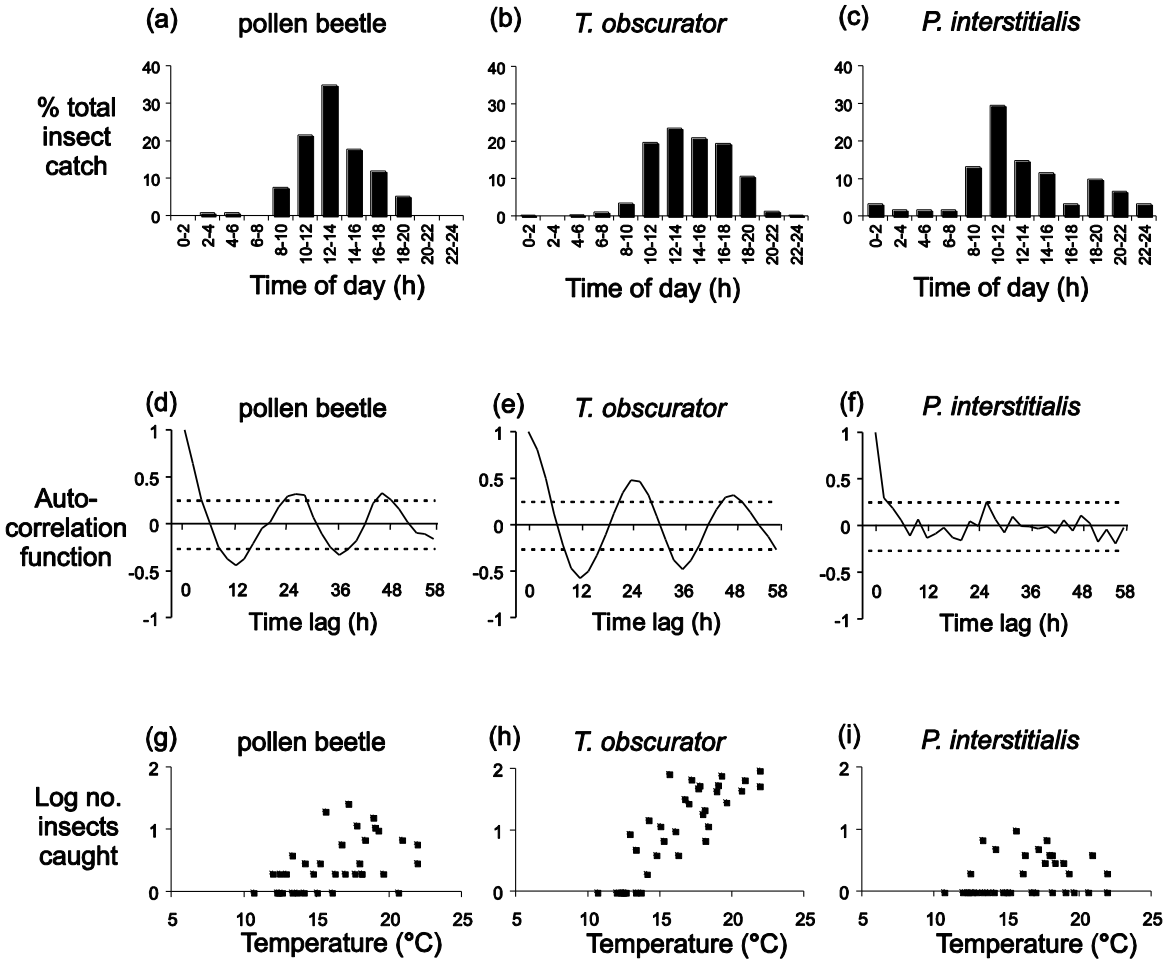

Figure 2. Diel distribution (a-c) and periodicity (d-f) of numbers of insects caught in Malaise traps and their relation with temperature during hours of daylight (g-l). Autocorrelation functions values above and below the dashed lines are significant. Each square on graphs (g-l) represents a 2 h sampling period.

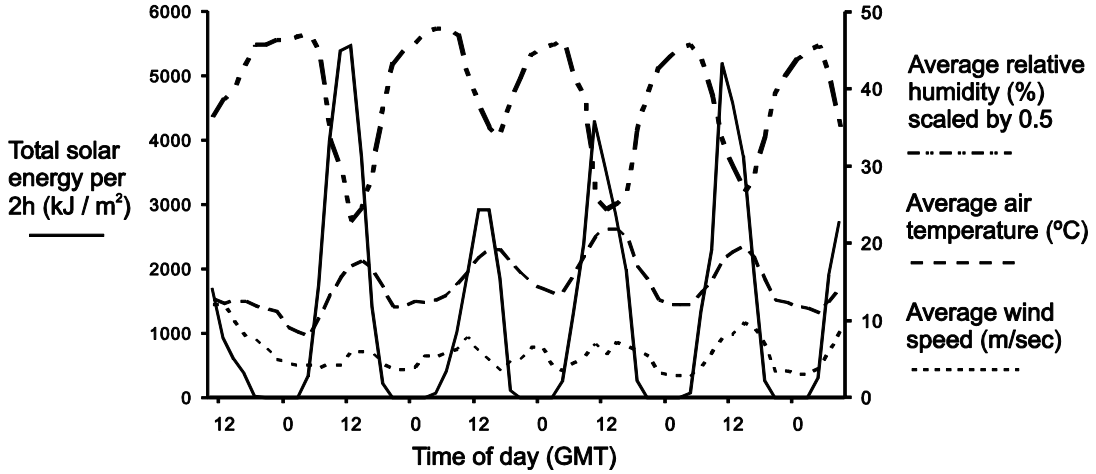

Figure 3. Meteorological data recorded during the five days that Malaise traps were operated.

Table 2. Cross correlations of insect numbers and meteorological variables at different time-lags.

Variable A	Lag of variable A (h)	Cross correlations of variable A with			
		Solar energy (kJ/m^2)	Average temperature (°C)	Relative humidity (%)	Average wind speed (m/sec)
No. of pollen beetle	0	0.68	0.52	-0.66	-0.28
	2	0.78	0.24	-0.34	-0.36
	4	0.48	0.18	0.12	-0.34
No. of *T. obscurator*	0	0.53	0.84	-0.80	-0.23
	2	0.72	0.67	-0.57	-0.43
	4	0.69	0.36	-0.21	-0.44
No. of *P. interstitialis*	0	0.63	0.24	-0.36	-0.36
	2	0.28	-0.10	-0.03	0.05
	4	0.07	-0.25	0.24	-0.57
Average temperature (°C)	0	0.48	-	-	-
	2	0.56	-	-	-
	4	0.40	-	-	-
Relative humidity (%)	0	-0.66	-0.73	-	-0.08
	2	-0.80	-0.57	-	0.18
	4	-0.68	-0.33	-	0.52

Discussion

This study succeeded in its objective of recording the seasonal and diel flight phenologies of pests and key parasitoid species at risk from insecticide applied in spring to winter rape in the UK. Malaise traps caught fewer insect species in sufficient numbers for analysis than did water traps, even though they were operated within the same period in the same crop. This may be in part due to the shorter duration of Malaise trapping. However, both types of trap are 'activity traps' in which numbers caught reflect not only the abundance of a species but its behaviour. Malaise traps may be more efficient than water traps at catching *T. obscurator* because they intercept flying insects from ground level to crop canopy height and this species seeks hosts in stems. By contrast, yellow water traps at crop canopy height may catch more species such as *T. heterocerus* that seek their hosts in yellow flowers in the canopy and catch fewer of the species that are active below the canopy.

Opportunities for temporal insecticide targeting to conserve parasitoids

Seasonal flight phenologies:

Pollen beetles immigrated into the crop at bud stage, the period when the crop is most at risk from pollen beetle damage and when, in the UK, control with insecticide is recommended should the established economic threshold be breached (Oakley, 2003). By contrast, virtually no pollen beetle parasitoids immigrated into the crop until flowering, by when the crop is rarely at risk. Thus strict adherence to recommendations to control pollen beetles at bud stage is likely to conserve pollen beetle parasitoids in the same way that pyrethroid sprays targeted at seed weevil at mid-flowering conserve their later immigrating parasitoid *T. perfectus* (Murchie *et al*., 1997). However, the activity in the crop of each parasitoid species was considerably longer than the period during which they emerged from the soil (Ferguson *et al*., 2003, 2006), leaving them vulnerable for extended periods to any application of insecticide to the crop. *Phradis interstitialis*, *T. heterocerus* and *T. obscurator*, the key parasitoids of the pollen beetle and the cabbage stem weevil were abundant in the crop at mid-flowering, the time recommended for application of pyrethroid insecticide to control cabbage seed weevil. There would appear to be no opportunity to change the date of insecticide application to conserve these parasitoids if the effectiveness of control is to be maintained and other means of insecticide targeting should be sought.

Diel periodicity of flight

Diel periodicity is the norm amongst insects, although the periodicity curve (distribution of activity over 24 h) varies, depending on whether the insect is nocturnal, diurnal or crepuscular (Lewis & Taylor, 1964). Each of the insects here was day-active, confirming earlier observations on *Meligethes* sp. and consistent with most parasitic Hymenoptera studied, including other Ichneumonidae (Lewis & Taylor, 1964). This probably reflects not only warmer daytime air temperatures but also the importance of visual cues in host location for both pest and parasitoid (Jönsson *et al.*, 2005; Cook *et al.*, 2006).

Influence of meteorological variables on insect flight

Numbers of *T. obscurator* and of pollen beetle caught in Malaise traps during daylight hours were correlated with solar energy and air temperature and were negatively correlated with RH. Air temperature and RH correlated most strongly with solar energy measured in the previous 2 h period and the correlations of *T. obscurator* and of pollen beetle numbers with solar energy showed the same time-lag. This suggests that the increased daytime activity of *T. obscurator* and pollen beetle is a direct response to temperature and/or RH and that the

response to solar energy is indirect through its effect on temperature and RH. The weak correlation of *P. interstitialis* numbers with temperature and with RH and lack of lag in their correlation with solar energy is in contrast with the other two insects and should be treated with caution because of the small number that were caught.

It is difficult to separate the influence of highly correlated meteorological variables on insect flight using an observational approach, or to determine the role of any endogenous circadian rhythms. However, air temperature is widely cited as the chief environmental determinant of insect flight (e.g. Taylor, 1963; Walters & Dixon, 1984) but RH can also have an important influence. Small insects with small haemolymph volumes are likely to be at greater risk from dehydration at low RH (Fadamiro & Wyatt, 1995) and may respond by remaining inactive in the boundary layer of vegetation where humidity is higher.

The relationship between temperature and flight by pollen beetle, as recorded by Malaise traps, accords with temperature thresholds for their immigration to crops reported elsewhere, although these can be rather variable (e.g. Nilsson, 1988; Sedivy & Kocourek, 1994). The same *c*. 14°C temperature threshold for flight appears to apply to *T. obscurator* and *P. interstitialis*, yet their immigration into the crop is later than that of the pollen beetle. The timing of spring immigration is dependent not only upon the flight temperature threshold but also upon the pre-existence of conditions that allow winter diapause to be terminated.

Catches of insects in Malaise traps were almost always weakly negatively-correlated with wind speed. Once an insect has located its host in a patchy environment, it is an advantage to avoid being blown away from it until the resource is exhausted. Similar responses have been shown in other ichneumonoid parasitoids, including *Aphidius* spp. which are of similar size to Tersilochinae (Juillet, 1964; Fink & Volkl, 1995; Schworer & Volkl, 2001).

Implications for conservation biological control

This study confirms that strict avoidance of the application of insecticide against pollen beetle when winter rape is in flower should avoid harm to pollen beetle parasitoids. However, it has demonstrated that the same parasitoids, together with those of the cabbage stem weevil, are present in the crop at mid-flowering and are therefore at risk from any insecticide applied to control the seed weevil at that time. Nevertheless, the parasitoids did not fly throughout daylight hours, probably limited by temperature. Parasitoids may be less at risk from insecticide if application were made in the early morning or late evening, when they rarely fly. Further work is needed to ascertain whether application at these times would reduce direct contact of spray droplets with parasitoids and reduce their mortality, or whether they would remain vulnerable to insecticide residues on the crop. Potential effects of such practice on the efficacy of insecticides against pests should also be tested. The studies described here will be extended to include more pest and parasitoid species and to better understand the relationship between insect flight and meteorological variables.

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Ceutorhynchus sp.

Mortality factors of the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Coleoptera, Curculionidae) in Europe

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Abstract: The cabbage seedpod weevil, *Ceutorhynchus obstrictus*, is a serious pest of canola and oilseed rape, *Brassica napus* and *B. rapa*, in North America and Europe. In Europe, several hymenopteran parasitoids are known to attack *C. obstrictus*, but the most important are the larval ectoparasitoids *Trichomalus perfectus* and *Mesopolobus morys* (Hymenoptera: Pteromalidae). These European parasitoids show the greatest potential for incorporation into an integrated pest management system for cabbage seedpod weevil in North America. Here we present a life table study that shows the actual impact of parasitoids, predators and other mortality factors on cabbage seedpod weevil populations in Europe.

Key words: cabbage seedpod weevil, mortality, life table

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The effectiveness of parasitoids in controlling the population size of *Ceutorhynchus assimilis* (Payk.) (Coleoptera: Curculionidae; syn. *C. obstrictus*) in winter oilseed rape

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Abstract: *Ceutorhynchus assimilis* (Paykull) (Coleoptera: Curculionidae) (cabbage seed weevil) is a widely distributed and major pest of oilseed rape in Europe. In Estonia, seed weevils are generally more abundant in winter oilseed rape and little is known about the occurrence, importance and efficiency of their parasitoids as biocontrol agents. In this four year study, the damage of *C. assimilis* and their larval parasitization rates were assessed in winter oilseed rape. Pod samples were collected from oilseed rape plants in 2004-2007 from three commercially grown, unsprayed winter oilseed rape crops at the pod maturation stage (BBCH 81-83) and incubated in emergence traps. Thereafter emerged larvae, their exit holes and parasitoids were counted, identified and the percentage of damaged pods and parasitism rate were calculated. Although the number of pods damaged by larvae of *C. assimilis* was relatively low, it increased continuously during the study years. The number of parasitizised larvae was lowest in 2005 and this was the only year when the parasitism rate decreased compared to the previous year. Notwithstanding this, the parasitization level showed generally a strong increase over the four years of the study and reached a notable 96% in 2007. The most abundant parasitoid was *Trichomalus perfectus* (Walker) (Hymenoptera: Pteromalidae). This study showed that parasitoids of *C. assimilis* were able to establish a viable population within the four years of the study, given suitable conditions. We conclude, that parasitoids of *C. assimilis* can efficiently control the population size of their host and that these natural enemies have a significant value for environmentally-friendly crop protection.

Key words: *Ceutorhynchus assimilis*, winter oilseed rape, larval parasitization rate

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Influence of insecticide application on host finding of the cabbage stem weevil parasitoid *Tersilochus obscurator* (Hym.; Ichneumonidae)

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Abstract: Host location of hymenopterous parasitoids is mainly based on olfactory cues emitted from the infested host plant or host larvae. In this study, we used behavioural bioassays to determine sub-lethal effects of insecticide residues on host finding of *Tersilochus obscurator* (Hym.; Ichneumonidae), a specialist parasitoid of the larvae of cabbage stem weevil, *Ceutorhynchus pallidactylus* (Mrsh.) that feeds within petioles and stems of oilseed rape. In Y-tube olfactometer experiments, *T. obscurator* females significantly preferred volatiles emitted from untreated leaves to volatiles emitted from leaves treated with the neonicotinoid insecticide thiacloprid, but not to volatiles emitted from leaves treated with the pyrethroid lambda-cyhalothrin. In dual-choice experiments females spent less time foraging on insecticide-treated leaves compared to untreated leaves or even avoided treated leaves. Further, on insecticide-treated leaves they performed less ovipositor probes than on untreated leaves. Thus, sub-lethal effects of insecticides may substantially reduce the level of parasitism of oilseed rape pests in the field.

Key words: *Ceutorhynchus pallidactylus*, larval parasitism, plant volatiles, insecticide, sub-lethal effects, oilseed rape

Introduction

Volatile compounds released from plants following attack by herbivorous insects are known to support host location by parasitic insects (Vet & Dicke, 1992). The composition and chemical identity of the volatile blend may provide specific cues to the herbivore species. Volatiles emitted from infested host plants, host larvae or frass might be particularly important for the location of endophagous host larvae hidden within the plant tissue. The larvae of the cabbage stem weevil, *Ceutorhynchus pallidactylus* (Mrsh.) (Col.; Curculionidae) feed concealed in the petioles and stems of oilseed rape (*Brassica napus* L.). They are host to the specialist larval endoparasitoid *Tersilochus obscurator* (Hym.; Ichneumonidae) (Ulber, 2003; Ulber *et al*., 2010). Parasitisation rates of *C. pallidactylus* larvae up to 50 % have been recorded from various European countries (Jourdheuil, 1960; Ulber, 2003). The frequent and widespread application of insecticides to oilseed rape may have lethal effects on adult parasitoids and reduce larval parasitism. In addition, even sub-lethal doses of insecticide residues on the plant might affect the level of parasitism by altering the volatile blend required for host finding (Haynes, 1988; Desneux *et al.*, 2007). In this study, behavioural analyses were conducted to determine sub-lethal effects of two insecticides, the pyrethroid lambda-cyhalothrin and the neonicotinoid thiacloprid, on host finding by *T. obscurator*.

Material and methods

Adults of *T. obscurator* were collected in April from unsprayed crops of oilseed rape by sweep net sampling and malaise trapping. In addition, adult parasitoids were reared from parasitized host larvae in the laboratory. Full-grown larvae of *C. pallidactylus* were collected from stems of unsprayed oilseed rape and transferred to plastic boxes containing moist soil substrate for pupation. After 10 weeks, the pupal cocoons of *T. obscurator* developing from parasitized hosts were sieved from the soil. The adult parasitoids emerging after hibernation were supplied with flowers of oilseed rape and moist cotton before use in the experiments.

Behavioural responses of adult *T. obscurator* to volatiles emitted from infested and uninfested leaves of oilseed rape, as well as from insecticide-treated and untreated leaves, both infested by larvae of *C. pallidactylus*, were studied by using Y-tube olfactometer bioassays (Takabayashi & Dicke, 1992) in the laboratory. Two separate 250 ml perspex vessels contained different leaves as odour sources. Clean air was pumped at 70 cm^3/sec through these vessels and introduced into the two arms of the olfactometer. Females of *T. obscurator* were released individually into the central tube and allowed to choose the preferred odour at the Y-junction. To stimulate phototactic movement of females, the light was directed from the back of the two arms of the olfactometer. Females not making a choice within 10 minutes were excluded from the analyses.

To study behavioural effects of insecticide spray residues on host location by *T. obscurator*, insecticide-treated and untreated leaves, both infested by larvae of *C. pallidactylus*, were offered to females for parasitization in dual-choice experiments. Females were released individually into ventilated perspex cages halfway between the two leaves and observed during a period of 5 minutes. The residence time females spent on each leaf and the percentage of ovipositor probes were recorded.

Results and discussion

In the first olfactometer bioassay *T. obscurator* females were given a choice between the odours of uninfested oilseed rape leaves and leaves infested by 2-6 L2-3 larvae of *C. pallidactylus*. Volatiles emitted from infested leaves were significantly preferred to volatiles emitted from uninfested leaves. Subsequently, the response of *T. obscurator* to the odour of insecticide-treated and untreated leaves, both infested by larvae of *C. pallidactylus*, was tested. Female *T. obscurator* showed a significant preference for volatiles emitted from untreated leaves as compared to volatiles emitted from leaves treated with the neonicotinoid-based insecticide thiacloprid. In contrast, no significant preference was found when the odour of untreated leaves was tested against the odour of leaves treated with the pyrethroid lambda-cyhalothrin.

In behavioural bioassays females spent less time foraging on thiacloprid-treated leaves compared to untreated leaves or even avoided the treated leaves. On insecticide treated leaves they performed less oviposition probings than on untreated leaves. Similar results have been obtained from other studies with parasitoids of aphids. Females of *Aphidius ervi* reduced their oviposition activity after contact with dry residues of lambda-cyhalothrin (Desneux *et al.*, 2003). Females of *Diaretiella rapae* spent less time resting on insecticide (pirimicarb, permethrin, malathion) treated Brussells' sprout plants than on untreated plants (Jiu and Waage, 1990). Further, when exposed to deltamethrin residues females of *Aphidius ropalosiphii* spent shorter visit times, groomed more frequently and rested less frequently than those on untreated plants (Longley and Jepson, 1996).

On-going studies will elucidate whether insecticide spray deposits on infested leaves inhibit host finding of *T. obscurator* females through alterations of volatiles emitted from these plants or by masking of specific plant volatiles needed for host location. Moreover, direct repellent effects of insecticide residuals on female parasitoids might also reduce the level of parasitism of *C. pallidactylus* by *T. obscurator*. Coupled GC-MS/EAD analyses are being conducted to provide evidence that *T. obscurator* females are able to discriminate between volatiles released from treated and untreated leaves.

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Effect on *Ceutorhynchus pallidactylus* abundance and damage of insecticide type and timing of treatment based on phenology of female immigration into oilseed rape crops

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Abstract: The effects of several pyrethroids and one combination of organophosphate and pyrethroid (alpha-cypermethrin, etofenprox, chlorpyrifos + cypermethrin) on *Ceutorhynchus pallidactylus* (Marsham, 1802), which was the predominating species in all years of assessment, and *Ceutorhynchus napi* (Gyllenhal, 1837), (Coleoptera: Curculionidae) were investigated under field conditions in the Czech Republic in 2006-2008.

The experimental dates of spraying each year were selected using results of monitoring the flight activity of both species using yellow water traps. The traps were located in the trial plots (at least 4 traps per plot) and also in the adjacent 1 ha area (36 traps positioned in a regular grid). The traps were emptied twice a week. The dates of spraying were (ideal treatment plan) as follows: 1) When the first adults of the monitored species appeared in the traps (as soon as possible after observation); 2) When the total number of adults caught approached or exceeded the Czech threshold values; 3) When the first females without eggs appeared in the traps in somewhat higher quantity (not only scattered individual females); 4) When a substantial proportion (approximately 50%) of the total number of females caught was ready to lay eggs (females with mature eggs).

Several assessments were conducted in each year. On each occasion, 20 plants per plot were sampled for: 1) The number of larvae in leaf stalks per plant, 2) The number of leaf stalks infested with larvae per plant, 3) the position of infested leaf stalks on plants, 4) the number of larvae per stem, 5) the total number of larvae per plant, and 6) the degree of stem infestation with larvae. The effects of treatments were compared for all mentioned assessments separately in each year of the study.

The occurence of *C. napi* was generally negligible and it was excluded from further analysis. There were significant differences between the effects of the compared insecticides on *C. pallidactylus* for the particular assessments in each year (2006-2008). Just the combination of chlorpyrifos + cypermethrin resulted in a remarkable decrease of the infestation levels in each of the three years. On the base of these results it is obvious that it was less important to establish the most suitable time of application (chlorpyrifos + cypermethrin) to obtain satisfactory results in comparison with the other treatments. The results of individually applied pyrethroids were not so clear. The effectivenesses of individually applied pyrethroids were significantly influenced by the time of spraying. The effects of the pyrethroids applied solo matched the effectiveness of the chlorpyrifos + cypermethrin treatments only when the time of spraying was optimal. The optimal time of spraying varied but occurred mostly when the first trapping of females appeared in traps in somewhat higher quantities and when the substantial proportion of the females were ready to lay eggs.

Key words: *Ceutorhynchus pallidactylus*, cabbage stem weevil, insecticide, alpha-cypermethrin, etofenprox, chlorpyrifos + cypermethrin, effectiveness

Pollen beetle

Using molecular methods to study pollen beetles: distribution and predation

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Abstract: We have studied genetic differentiation among populations of pollen beetles in Sweden and in Europe using Amplified Fragment Length Polymorphism (AFLP) analysis. For Swedish populations there was a high level of genetic variation within populations and a high level of gene flow among populations. European populations showed regional diversification and a low level of gene flow. Another molecular technique we are currently investigating is the use of PCR to detect pollen beetle DNA in selected predators. We hope to gain more information about the timing and frequency of predation events for a variety of predators.

Key words: pests, pollen beetles, genetic variation, gene flow, AFLP

Resistance of pollen beetle (*Meligethes aeneus* F.) to pyrethroids – results of a national monitoring in Luxembourg

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Abstract: Following reports about severe resistance problems for pyrethroids to control the pollen beetle (*Meligethes aeneus* F.) in oilseed rape in several European countries, the efficacy of the type II pyrethroids have been tested on 27 populations of the pollen beetle in Grand Duchy of Luxembourg. Cases of resistance to type II pyrethroids have been found on 11 locations. The level of resistance cannot be described as moderate anymore, since populations showed resistance at normal field application rate. Most of the resistant populations have been found near borders with neighbouring countries, e.g. Canton Redange (near Belgium), Canton Remich (near France) and Cantons Grevenmacher (near Germany). Two populations of showing a high level of pyrethroid resistance have been found in isolated places, surrounded by wooded area. Additionally, tests with the type I pyrethroid Bifenthrin and organophosphate Chlorpyrifos-methyl demonstrated that these active substances are still efficient on resistant populations for the moment. The results of this study suggest the hypothesis that an increase in pyrethroid resistance can be expected in the coming years in Luxembourg.

Key words: Pollen beetle, populations, pryrethroid II resistance

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First results of monitoring the occurrence of resistant pollen beetles (*Meligethes aeneus* Fabricius 1775) in the Czech Republic

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Abstract: Laboratory experiments were conducted to test effects of the active substances of two different pyrethroids on imagos of the pollen beetle (*Meligethes aeneus* Fabricius 1775) collected in various areas of the Czech Republic in 2008. The tested pyrethroids were: lambda-cyhalothrin as a specimen of the ester pyrethroids (type II) and etofenprox as a specimen of the other group of pyrethroids (type I, ether pyrethroids). An adult-vial-test (IRAC Susceptibility Test Method No. 11) was used.

The pyrethroids were applied in the glass tubes (internal surface area: 37.97 cm^2). The lambda-cyhalothrin was applied as four doses: 0 µg cm^{-2} (acetone only); 0.003 µg cm^{-2} (4% of the European field application rate of 7.5 g a.i. ha^{-1}); 0.015 µg cm^{-2} (20% rate); 0.075 µg cm^{-2} (100% rate). The etofenprox was also applied as four doses: 0 µg cm^{-2} (acetone only); 0.016 µg cm^{-2} (4% of the Czech field application rate of 40 g a.i. ha^{-1}); 0.080 µg cm^{-2} (20% rate); 0.400 µg cm^{-2} (100% rate). The collected insects were exposed to the dried residues of the insecticides for up to 24 hours. The response of beetles were assigned into one of four categories in accordance to their reactions to the insecticidal residues after 1, 5 and after 24 hours from the beginning of the tests. The active imagos without any symptoms of insecticide affect were assigned to category 4, imagos with slight symptoms (retained ability to move the legs) were assigned to category 3, severely affected beetles (immobility, tremor) were assigned to category 2 and dead imagos were assigned to category 1.

Significant differences were found among the reactions of the pollen beetles originating from the compared localities to lambda-cyhalothrin applied at the 100% rate. The differences in the lambda-cyhalothrin effectiveness expressed in accordance to Abbott exceeded 50% among the different localities. Conversely, there were no significant differences between the reactions of the pollen beetles originating from the five compared localities to the etofenprox applied at the 100% rate. The differences in the etofenprox effectiveness expressed in accordance with Abbott (and in the pollen beetles mortalities) were minor between the compared localities. On the base of other analyses, it is also possible to conclude that the localities where the beetles showed significantly different reactions to the lambda-cyhalothrin were not different in this aspect concerning the etofenprox.

Key words: *Meligethes aeneus*, pollen beetle, insecticide resistance, lambda-cyhalothrin, etofenprox, adult-vial-test

Introduction

In the Czech Republic, not only are the type II pyrethroids (ester pyrethroids which are halogen substituted and contain an α-amino group) available for the control of pollen beetles

(*Meligethes aeneus* Fabricius 1775) in oilseed rape but they are the most used group of insecticides for a very long time. Therefore resistance development of the pest to type II pyrethroids is now a very serious issue. Also, due to the fact that exact experimental results (particularly from the field, but even from the laboratory) are quite scarce and ambiguous in the country. In recent years *Meligethes aeneus* has developed resistance to pyrethroids in different European regions (Ballanger *et al.*, 2003; Derron *et al.*, 2004; Hansen, 2003; Wegorek, 2005; etc.) and have been particularly well monitored in Germany (Nauen, 2005; Thieme and Hoffmann, 2006; Heimbach *et al.*, 2006, Glattkowski *et al.*, 2008; Muller *et al.*, 2008). Hence it is relevant and high time to begin with the monitoring of the distribution of pollen beetles populations with decreased susceptibility to pyrethroids in using uniform methodologies and under a common sampling plan through the whole of the Czech Republic. In this paper the first results of testing *M. aeneus* samples originating from various localities in the Czech Republic are presented. The test method used (Method No. 11) is recommended by the Insecticide Resistance Action Committee. The main object of the study was to find out if pollen beetle populations resistant to lambda-cyhalothrin are present in the Czech Republic. We also tested the effects of etofenprox (a type I pyrethroid). The type I pyrethroids should be less prone to pyrethroid resistance, which is thought to be metabolic in nature. The differences between the effects of the two tested active substances are discussed.

Material and methods

The laboratory experiments were conducted to test effects of two different pyrethroids on imagos of pollen beetles (*Meligethes aeneus* Fabricius 1775) sampled in various areas of the Czech Republic in 2008. The tested pyrethroids were: lambda-cyhalothrin as a member of the ester pyrethroids group (type II) and etofenprox as a member of the other group of pyrethroids (type I, ether pyrethroids). As the test method an adult-vial-test (IRAC Susceptibility Test Method No. 11) was used. The pyrethroids were applied in the glass tubes (internal surface area: 37.97 cm^2). The lambda-cyhalothrin (analytical sample from Syngenta Crop Protection AG, Basel, Switzerland) was applied in four doses: 0 $\mu g\, cm^{-2}$ (acetone only); 0.003 $\mu g\, cm^{-2}$ (4% of the European field application rate of 7.5 g a.i. ha^{-1}); 0.015 $\mu g\, cm^{-2}$ (20% rate); 0.075 $\mu g\, cm^{-2}$ (100% rate). The etofenprox (analytical sample from Mitsui Chemicals, INC., Minato-Ku Tokyo, Japan) was also applied in four doses: 0 $\mu g\, cm^{-2}$ (acetone only); 0.016 $\mu g\, cm^{-2}$ (4% of the Czech field application rate of 40 g a.i. ha^{-1}); 0.080 $\mu g\, cm^{-2}$ (20% rate); 0.400 $\mu g\, cm^{-2}$ (100% rate). The sampled insects were exposed to the dried residues of the insecticides for up to 24 hours. The numbers of individuals were categorized into one of four categories (= infliction degrees) according to their responses to the insecticidal residues after 1, 5 and 24 hours from the beginning of the tests. The active imagos without any symptoms of infliction were assigned into category 4, imagos with slight symptoms of infliction (slight dis-coordination but retained ability to move their legs) were assigned into category 3, severely affected beetles (obvious dis-coordination, immobility, tremors) were assigned into category 2 and dead imagos were assigned into category 1.

Laboratory assessments were conducted in three workplaces:

1) Šumperk (AGRITEC, Research, Breeding & Services Ltd.): 15 samples for lambda-cyhalothrin testing and 5 samples for etofenprox testing
2) Opava (OSEVA PRO Ltd., Filial establishment of the Research Institute of Oilseed Crops at Opava): 10 samples for lambda-cyhalothrin testing
3) Troubsko u Brna (Research Institute of Fodder Crops, Ltd.): 5 samples for lambda-cyhalothrin testing.

Results and discussions

Effects of lambda-cyhalothrin (100% dose; adult-vial-test)

We did not record any fully susceptible sample during the monitoring from Šumperk (Table 1). However, remarkable differences among the 15 samples of *M. aeneus* gathered from various localities in the course of May and June 2008 (6.5.-30.6.) were found in their reactions to lambda-cyhalothrin (l.c.). On the base of *Meligethes* imagos' reactions which were observed always 5 hours from the beginning of the tests, we can conclude that the mean infliction degree ranged between the values 3.17 and 1.77. It means the least susceptible sample (locality 6) was on average composed of the beetles whose grouped reaction to the 100% dose of l.c. moved between the infliction degrees 3 and 4 (slightly afflicted individuals in average). The least susceptible sample came from a commercial crop near to the village of Chromeč. The beetles were sampled on 7 May on oilseed rape (flowering stage). On the contrary, the most susceptible sample (locality 8) was composed of the beetles whose average reaction to 100% dose of l.c. moved between the infliction degrees 1 and 2 (dead and hardly afflicted beetles = beetles without any ability to do further damage to plants). The most susceptible sample came from the locality Blučina nearby Brno (a commercial oilseed rape crop; term of sampling: 28 May; end of flowering stage). From the fifteen samples there were only four (localities 7, 8, 12, 15) with a mean infliction degree below the 2.30 value and only one sample with a mean infliction degree below the 2.00 value (second column; Table 1). Most of the samples fall into the categories 2 to 3.

The real mortalities (column 3 in Table 1) recorded 5 hours after the beginning of the experiment were lower than we expected. However, there were significant differences among the mean values (Tukey test; $P < 0.05$). Samples 7 and 8 seem to be somewhat more susceptible than the others. There were significantly lower values of mean mortality in samples 1, 2, 3, 4 and 5 in comparison with sample 7.

From the comparison of the mean proportions of dead + hardly afflicted beetles (column 4 in Table 1) the significant differences among the compared samples emerges again (Tukey test; $P < 0.05$). There were four samples among the others which contained even less than 40% of individuals fully excluded from other harmful activities after treatment with 100% dose. In two samples (sample 6 and 9) the proportions of dead + severely afflicted beetles were only 20-30%. On the contrary there were also samples recorded with relatively high proportions (above 70%) of imagos fully excluded from other harmful activities after the treatment (samples 5, 7, 8, 12 and 15). However, we didn´t observe any sample of *Meligethes* imagos fully susceptible to l.c. in this sense.

It is obvious from the results of the comparison of the proportions of fully active individuals (column 5, in Table 1) that the samples were relatively different even in this trait (Tukey test; $P < 0.05$). The highest proportions of fully active individuals 5 hours after the treatment were recorded from samples 4, 6, 9, 10 and 14 (30% and more). Sample 6 seemed to be the least susceptible (50 % of fully active imagos).

Table 1. Comparison of pollen beetles (from 15 different localities) reactions exposed to 100% doses of lambda-cyhalothrin after 5 hours from the beginning of the test (Šumperk, 2008)

No. of locality (sample)	Mean infliction degree[a,b]	Mean value of mortality (%)[b]	Mean proportion of dead + severely afflicted individuals (%)[b]	Mean proportion of fully active individuals (%)[b]
1	2.60 abc	3.33 cd	56.67 bcde	20.00 ab
2	2.93 ab	0.00 d	32.73 cde	25.76 ab
3	2.50 abc	10.00 bcd	56.67 bcde	16.67 ab
4	2.83 ab	10.00 bcd	40.00 bcde	30.00 ab
5	2.32 abc	0.00 d	77.78 ab	9.44 ab
6	3.17 a	13.33 abcd	20.00 e	50.00 a
7	2.07 bc	43.33 a	73.33 abc	23.33 ab
8	1.77 c	33.30 ab	89.53 a	0.00 b
9	2.80 ab	20.00 abc	30.00 de	30.00 ab
10	2.43 abc	26.67 ab	63.33 abcd	33.33 a
11	2.56 abc	15.71 abc	57.62 abcde	26.19 ab
12	2.27 bc	16.67 abc	73.33 abc	16.67 ab
13	2.40 abc	13.33 bcd	66.67 abcd	20.00 ab
14	2.50 abc	16.67 abc	66.67 abcd	33.33 a
15	2.30 bc	16.67 abc	76.67 ab	23.33 ab

[a] the individual imagos were grouped into one of four categories (= infliction degrees) in accordance with their reactions to lambda-cyhalothrin residues (dead imagos into category 1, severely afflicted imagos into category 2, slightly afflicted imagos into category 3 and fully active imagos – without any visible infliction – into category 4; there are weighted averages of the counts in the table)
[b] the numbers marked with differing letters are significantly different (Tukey test; $P < 0.05$)
Localities of M. aeneus sampling: 1) Zábřeh na Moravě; 2) Šumperk Agritec; 3) Senice na Hané; 4) Hradec nad Svitavou; 5) Náměšť nad Oslavou (Vicenice); 6) Chromeč; 7) Libina; 8) Blučina – Brno; 9) Bludov; 10) Libivá; 11) Šumperk Agritec II; 12) Uničov; 13) Skrbeň; 14) Rapotín I; 15) Rapotín II

We recorded one fully (Kunín 14.5.08) and two almost fully (Kujavy 13.5.08; H. Životice 20.5.08) *lam*bda-cyhalothrin susceptible samples among the ten subpopulations tested in Opava. However, there were also several samples with markedly decreased susceptibility to the active substance. The least sensitive subpopulations (Opava I; Opava III; Opava IV) came from the surrounding area of the town Opava. In general they showed remarkable differences (Tukey test; $P < 0.05$) among the 10 samples of *M. aeneus* taken from various localities and tested in Opava (12.5.-17.6.) to their reactions to 100% doses of lambda-cyhalothrin 5 hours after the treatment (Figure 1).

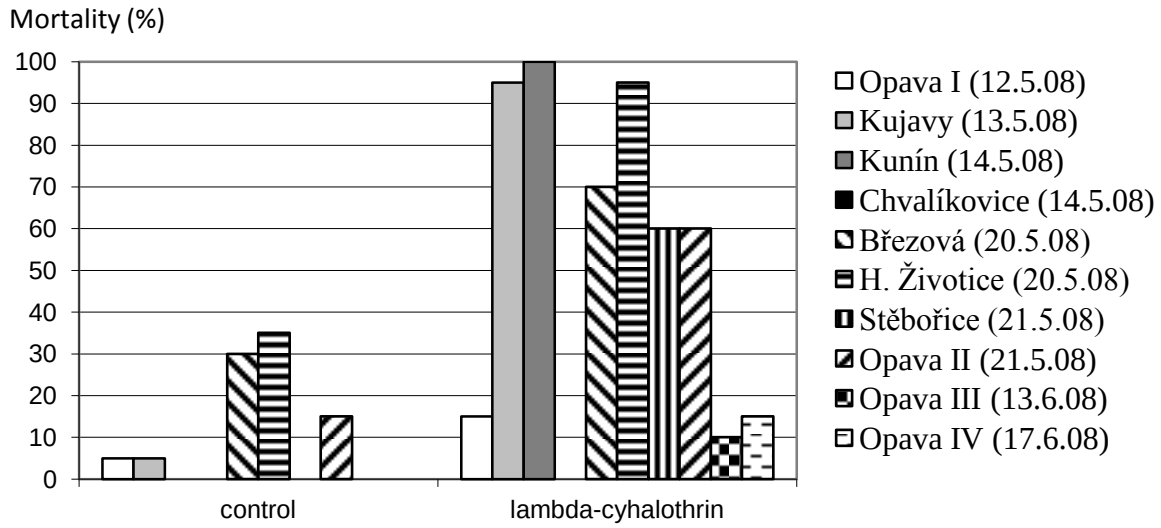

Figure 1. Effects of lambda-cyhalothrin on pollen beetles sampled from 10 different localities (adult-vial-test; 100% doses; assessment 5 hours after treatment; Opava, 2008)

The results from Troubsko (sampled during June 2008) show obvious differences among the calculated values of lambda-cyhalothrin effectiveness (100% dose; 5 hours after treatment) between the compared samples (5 sampling localities) (Figure 2).

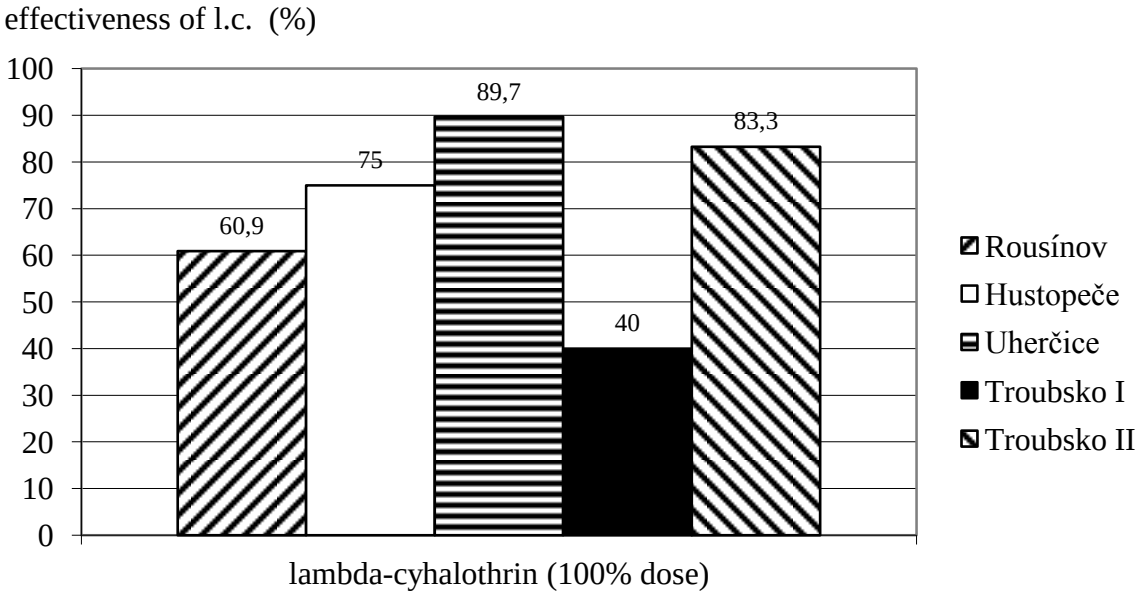

Figure 2. Effectiveness of lambda-cyhalothrin expressed in according to Abbott (control = 0%) on the samples originated from five different localities (adult-vial-test; 100% doses; 5 hours after treatment; Troubsko, 2008)

Effects of etofenprox (100% dose; adult-vial-test)

From the results listed in table 2, it is obvious that the level of susceptibility of *Meligethes* imagos to etofenprox (et.) (100% dose) was substantially higher in comparison with l.c. effects (100% dose; Table 1). The mean infliction degree for the five tested samples ranged between 1.76 and 1.97. There were no significant differences among the samples in this trait (Tukey test). It is interesting that the levels of mortality (column 3 in Table 2) were relatively low (6.67-26.19) for all tested samples (non-significant differences: Tukey test; $P < 0.05$). Hence most of the imagos fell into the category severely afflicted – the individuals were not able to move but they were not dead (column 4 in Table 2). The mean proportions of dead + severely afflicted individuals were relatively high in general (90.74-100%). Meaning 5 hours after treatment with a 100% dose of etofenprox most of the beetles were not able to do further damage to plants any more. There were no significant differences among the compared samples in this trait (Tukey-test; $P < 0.05$).

Fully active individuals (unafflicted) were not recorded from any sample five hours after the treatment.

Table 2. Comparison of pollen beetles (from 5 different localities) reactions exposed to 100% doses of etofenprox after 5 hours from the beginning of the test (Šumperk, 2008)

locality	Mean infliction degree[a,b]	Mean value of mortality (%)[b]	Mean proportion of dead + severely afflicted individuals (%)[b]	Mean proportion of fully active individuals (%)[b]
1	1.93 a	6.67 a	100.00 a	0.00
2	1.77 a	23.33 a	100.00 a	0.00
3	1.76 a	26.19 a	100.00 a	0.00
4	1.97 a	12.73 a	90.00 a	0.00
5	1.88 a	21.11 a	90.74 a	0.00

[a] the individual imagos were grouped into one of the four categories (= infliction degrees) in accordance to their reactions to etofenprox residues (dead imagos into category 1, severely afflicted imagos into category 2, slightly afflicted imagos into category 3 and fully active imagos – without any visible infliction – into category 4; there are weighted averages of the counts in the table)

[b] the numbers marked with the differing letters are significantly different (Tukey test; $P < 0.05$)

Localities of *M. aeneus* sampling: 1) Zábřeh na Moravě; 2) Senice na Hané; 3) Šumperk Agritec; 4) Náměšť nad Oslavou (Vicenice); 5) Hradec nad Svitavou

Differences between the effectiveness of lambda-cyhalothrin and etofenprox (adult-vial-test)

The reactions of pollen beetle imagos to 100% doses of the two compared pyrethroids were substantially different (Figure 3). The effects of lambda-cyhalothrin were much more variable in relation to the sampling locality in comparison with etofenprox effects. The effects of etofenprox were quite uniform in this sense regardless of the efficacy failures of lambda-cyhalothrin.

The differences between the effectiveness of the two compared active substances (100% doses, 5 hours after treatment) expressed according to Abbott are shown in Figure 4. In general, the etofenprox was more effective than lambda-cyhalothrin. The effectiveness of etofenprox was probably not influenced by the recorded levels of resistance to lambda-

cyhalothrin of the samples tested simultaneously (Zábřeh na Moravě; Šumperk Agritec; Senice na Hané; Náměšť nad Oslavou (Vicenice); Hradec nad Svitavou).

Figures 5 and 6 show the proportions of dead + severely afflicted beetles of two samples tested together (Vicenice; Šumperk Agritec) plotted against the dose-rate of both insecticides assessed. The lambda-cyhalothrin curves differ remarkably especially at the 20 and 100% doses. However, the etofenprox curves also vary. The differences at the lower doses are distinct. These findings suggest future problems even with etofenprox at the localities with the occurrence of pollen beetles with proved lower susceptibility to the type II pyrethroids. Higher doses of etofenprox can still reliably give control but control with lower doses (4%; 20%) of the active substance can already break down there.

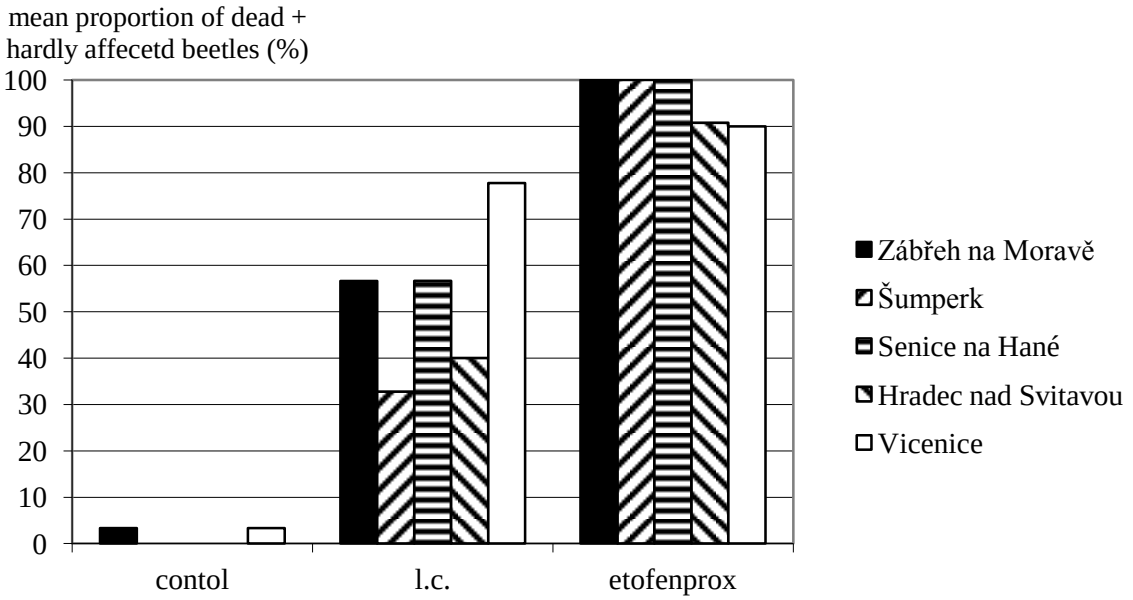

Figure 3. Comparison of lambda-cyhalothrin and etofenprox effects on pollen beetles sampled on five different localities (adult-vial-test; 100% doses; assessment 5 hours after treatment; Šumperk, 2008)

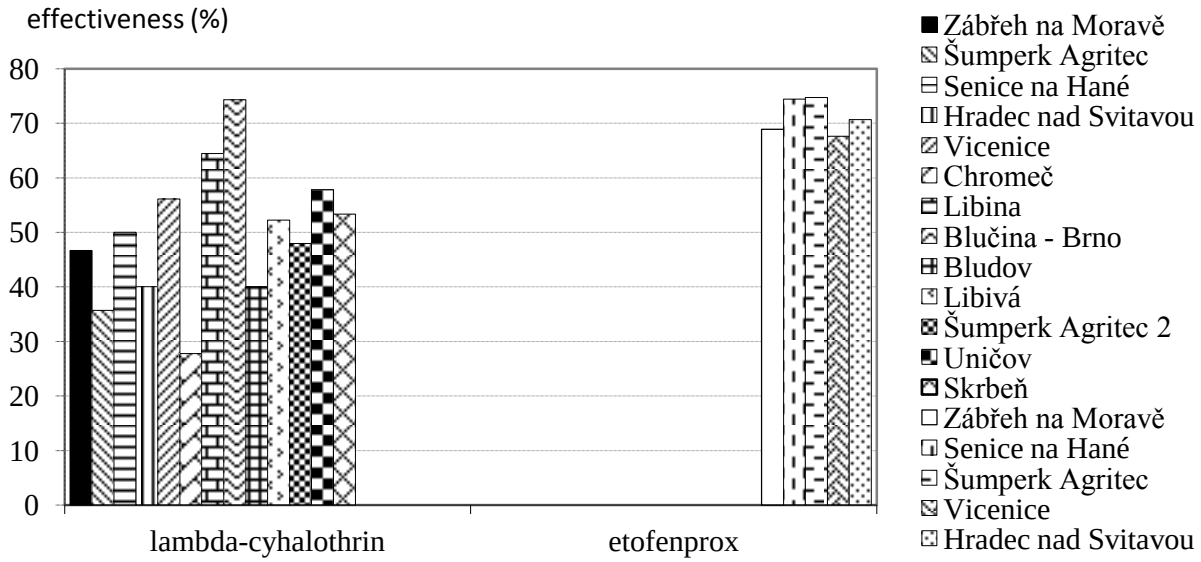

Figure 4. Differences in the effectiveness of the two compared active substances expressed in accordance to Abbott (control = 0%) (adult-vial-test; 100% doses, 5 hours after treatment; Šumperk, 2008)

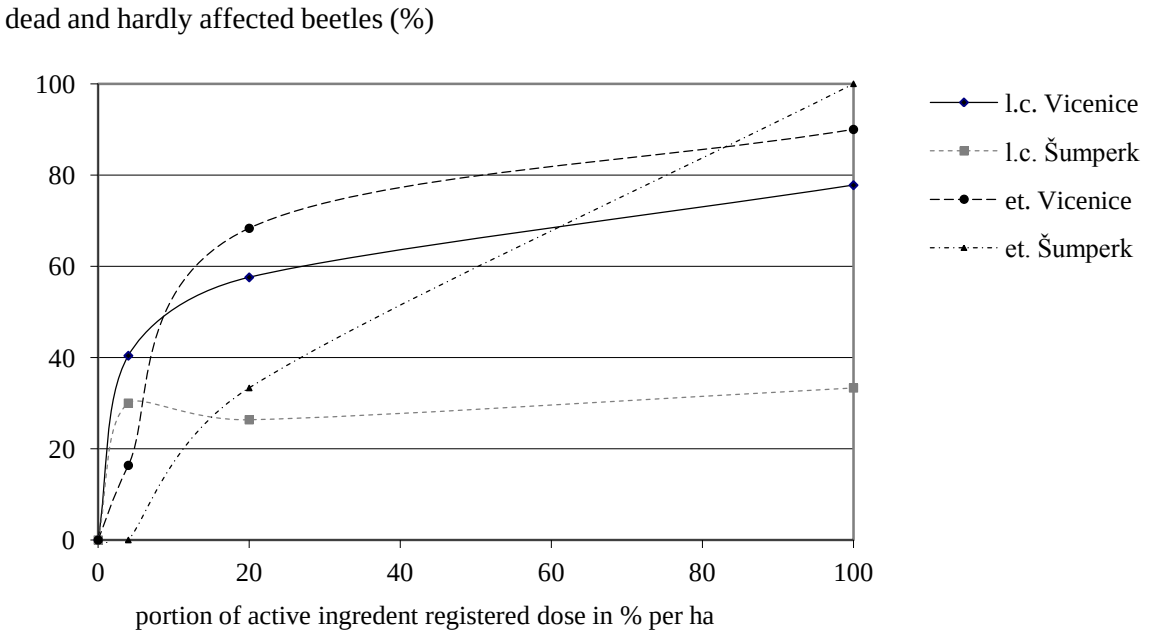

Figure 5. Differences in reactions of *Meligethes aeneus* to lambda-cyalothrin and etofenprox from two localities diverging in levels of resistance to lambda-cyhalothrin (adult-vial-test; 5 hours after treatment; Šumperk, 2008)

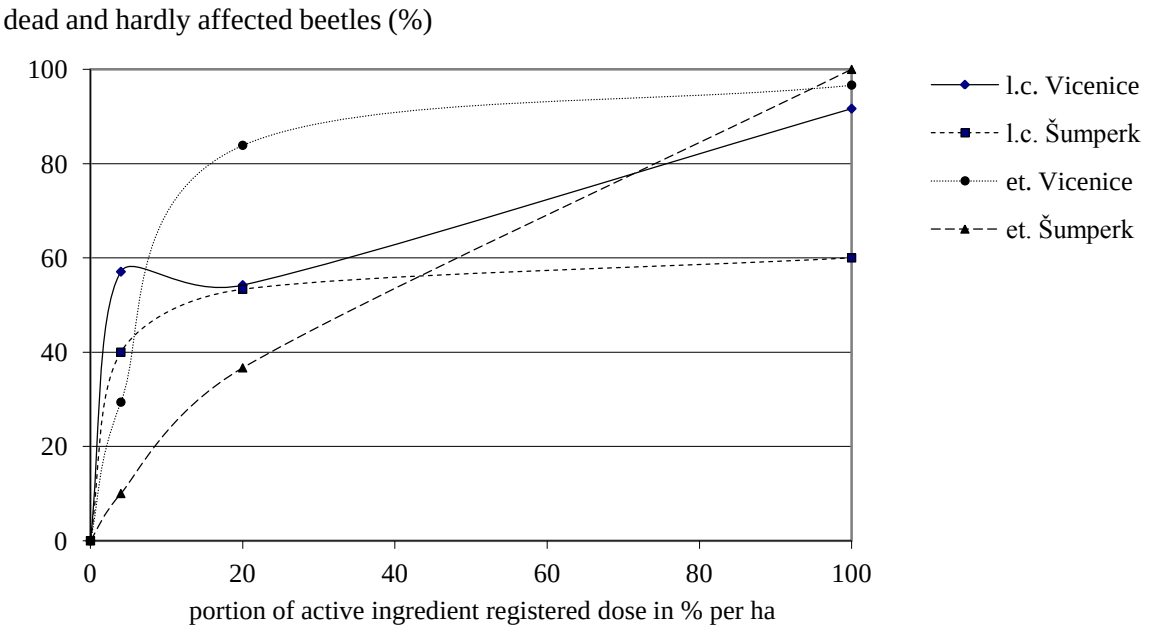

Figure 6. Differences in reactions of *Meligethes aeneus* imagos to lambda-cyalothrin and etofenprox from two localities diverging in levels of resistance to lambda-cyhalothrin (adult-vial-test; 24 hours after treatment; Šumperk, 2008)

Conclusions

1) The occurrence of *M. aeneus* subpopulations with markedly decreased susceptibility to lambda-cyhalothrin was proved from most of the tested localities in the Czech Republic in 2008.
2) The tested *M. aeneus* subpopulations showed significant differences among their reactions to lambda-cyhalothrin.
3) Fully susceptible subpopulations of *M. aeneus* to lambda-cyhalothrin were almost non-existent among the tested samples in 2008.
4) The effectiveness of etofenprox applied at the highest doses (100%) was probably not influenced by the level resistance to lambda-cyhalothrin.
5) Decreased effects of etofenprox applied at lower doses (4%; 20%) to samples of *Meligethes* with high levels of resistance to lambda-cyhalothrin indicates the high possibility of future problems with effective control even with this active substance.

Acknowledgements

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Do insecticide resistant pollen beetles suffer a higher mortality during hibernation?

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Abstract: Pollen beetles are common pests of oilseed rape. There are only a few products registered for their control and for many years only pyrethroids were permitted. As a consequence selection for resistance to pyrethroids in pollen beetle populations has occurred. Although the biology of the pollen beetle has been widely studied in oilseed rape, there is little knowledge on its hibernation sites and mortality during hibernation. Therefore it was unknown whether pollen beetles colonizing oilseed rape fields in spring after hibernation exhibit the same level of resistance as they had the previous year.

In winter 2007/2008, soil samples were collected at different times from different locations in Bavaria, Brandenburg and Mecklenburg-Western Pomerania in Germany. The beetles were extracted alive from these samples in the laboratory, and their susceptibility to different insecticides, including pyrethroids, was analysed and compared with that of beetles emerging from hibernation and colonizing oilseed rape fields the following spring.

Key words: Pollen beetles, populations, insecticide susceptibility

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Phenotypic search for promising entomopathogenic fungal isolates to control pollen beetles

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Abstract: Twenty five isolates of entomopathogenic fungi belonging to the species *Beauveria bassiana* (Balsamo) Vuillemin (21), *B. brongniartii* (Saccardo) Petch (2), *Paecilomyces fumosoroseus* (Wize) (1) and *Metarhizium anisopliae* Sorokin (1) were screened for virulence against pollen beetle *Meligethes aeneus*. Several of the selected *B. bassiana* isolates originated from naturally infected pollen beetles collected in Switzerland. For the laboratory bioassays adult pollen beetles were dipped into a conidial suspension of 1 x 10^7 spores per ml (0,05% Tween 80) and incubated at 22 °C, 70% rh, L:D 16:8. Infection rates were recorded after 15 days. Seven isolates of *B. bassiana* as well as the *P. fumosoroseus* isolate achieved infection rates of ≥ 67%. Twelve isolates caused intermediate infection rates of 34-66%, whereas five *B. bassiana* isolates showed infection rates of less than 33%.

Key words: biocontrol, insect parasitic fungi, virulence screening, *Meligethes* sp., *Ceutorhynchus* sp.

Introduction

The pollen beetle, *Meligethes aeneus*, is the most important pest of winter oilseed rape in Switzerland and can cause substantial yield losses both in conventional and organic oilseed rape production. In the last few years, insecticidal resistance has been repeatedly reported from Switzerland (Derron *et al.*, 2004) and presents new challenges for resistance management. In organic farming, no pest control measures are actually available.

Our research projects attempt to develop alternative pest control strategies both to reduce insecticide use and to prevent propagation of resistance as well as to provide environmentally friendly and potent pest control solutions to organic oilseed rape producers.

The aim of the present study was to evaluate the potential of selected entomopathogenic fungal isolates for the biological control of pollen beetles.

Material and methods

Fungal isolates

Twenty five isolates of entomopathogenic fungi from the Agroscope ART collection were screened for virulence against adult pollen beetles (*Meligethes* sp.). The selected fungi originated from different host insects. Eighteen isolates originated from pollen beetles collected in Switzerland and one each from the European corn borer *(Ostrinia nubilalis)*, the Western corn root worm (*Diabrotica virgifera virgifera*), the cockchafer (*Melolontha melolontha*), the codling moth (*Cydia pomonella*), the bark beetle (*Ips typographus*), an unidentified carabid beetle and a soil sample, respectively.

Pollen beetles
Pollen beetles were collected in an organic oilseed rape field close to the research institute and kept with buds and pollen until use within 24 h.

Laboratory bioassays
Ten adult pollen beetles each were dipped into a conidial suspension (1 x 10^7 c./ml; 0.05% Tween80) in a spherical tea strainer for 5 seconds and incubated in a petri dish at 22 °C, 70% rh, L:D 16:8. For each fungal isolate three replicates were performed. Infection rate was measured after 15 days.

Results and discussion

All four species of entomopathogenic fungi were able to infect and kill adult pollen beetles. *Beauveria bassiana* was most promising and achieved high infection rates for several isolates (Figure 1). Natural infections of pollen beetle populations with *B. bassiana* were reported from Switzerland by Pilz and Keller (2006). However, in the present study fourteen out of eighteen *B. bassiana* isolates originating from pollen beetles showed low or intermediate virulence, while four isolates proved to be promising. The selected *P. fumosoroseus* and *M. anisopliae* strains achieved 73.9% and 63.9% infection rate, whereas *B. brongniartii* only infected about half of the exposed pollen beetles.

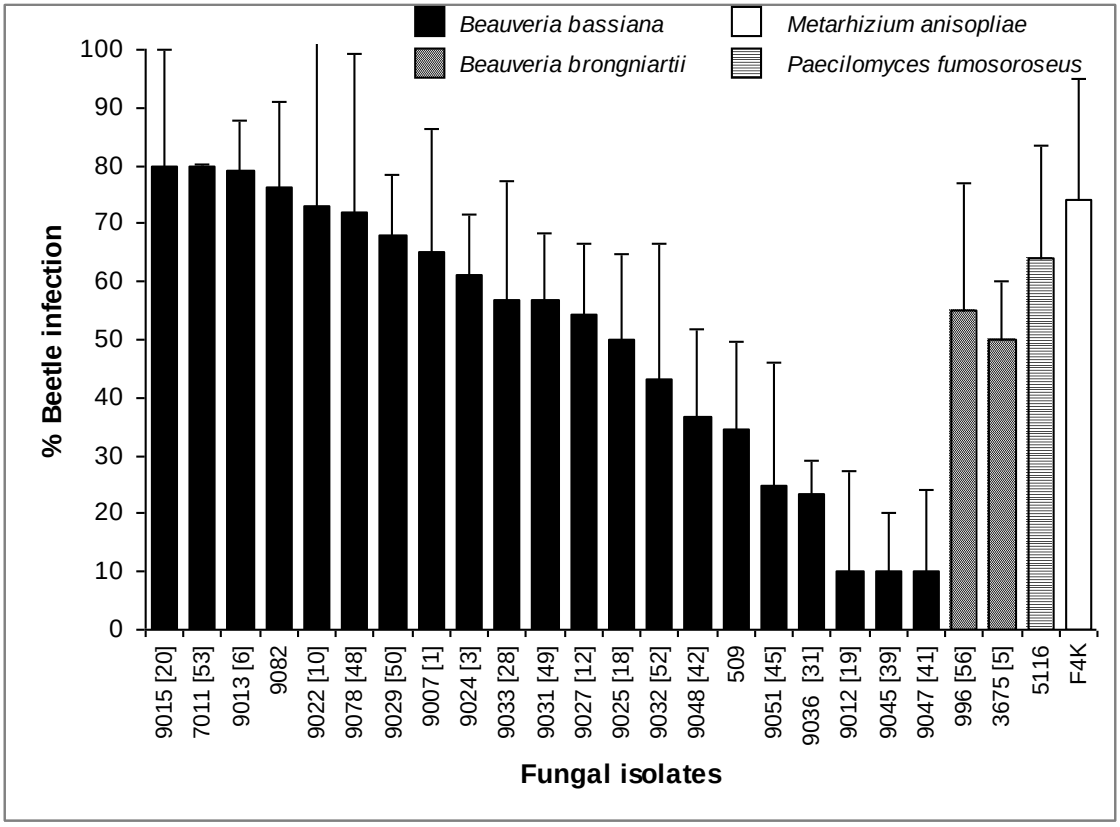

Figure 1. Pollen beetle infection by entomopathogenic fungal isolates.

With regard to practical application, additional parameters such as temperature activity and host range were investigated for selected isolates. At temperatures below 15 °C beetle mortality and fungal infection were considerably reduced. Desirable side-effects on other important oilseed rape pests were investigated as well. The locally important *M. viridescens* was susceptible to *B. bassiana*, as well as the rape stem weevil *Ceutorhynchus napi*, and the cabbage stem weevil *C. quadridens*. It was further shown that both mortality and infection rate can be significantly increased using oil-based formulations.

The present study revealed that Swiss entomopathogenic fungi show good potential for practical use in the biological control of important oilseed rape pests. Strains originating from pollen beetles were among the most promising isolates tested. Additional investigations are needed to confirm the biocontrol potential under field conditions. Besides the selection of the most promising fungal isolates, both product formulation and application timing and technique could also be improved step by step.

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Effects of tillage system and larval parasitism on pollen beetle (*Meligethes* spp.) populations

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Abstract: The abundance of pollen beetle (*Meligethes aeneus* F.) populations varies greatly between years. Weather conditions, area of oilseed rape grown, duration of bud and flowering stages, overwintering mortality and biological control by natural enemies have been identified as contributing factors to these variations. Furthermore, crop management practices such as insecticide application and tillage regime may also impact the population dynamics of pollen beetle. Soil management practices in particular might affect the survival of this pest during the time of development from the larval to the teneral stage within the soil.

The abundance of adults and larvae of pollen beetle was recorded over three years in a long-term field experiment. Plots were subjected throughout the rotation of winter wheat, winter barley and winter oilseed rape to three different tillage regimes (i) conventional tillage: ploughing to 25 cm depth, furrow roller, rotary harrow; (ii) conservation tillage: rototiller to 10 cm depth; (iii) no till: direct drilling into the cereal stubble. In April/May, the abundance of the overwintered generation was determined by repeated counts of the number of pollen beetle on the plants. Mature pollen beetle larvae dropping from flowers to pupate in soil were captured in water trays placed below the crop canopy. The emergence of new generation beetles was assessed by using ground-photoeclectors. Percentage larval parasitism was determined by dissection of full-grown larvae.

In all years, the abundance of the over-wintered generation beetles on the crop showed little difference between the conventional tillage, conservation tillage and no till plots. The number of mature larvae per m² was significantly reduced only in conventional tillage plots in 1997. Consistently higher numbers of new generation beetles emerged from conventional tillage plots as compared to conservation tillage plots and no till plots. In proportion to the number of mature larvae dropping to the ground, the mean rate of beetles emerging from conventional tillage, conservation tillage and no till plots was 9.2%, 7.5% and 4.3%, respectively.

The larval parasitism of *Meligethes* spp. by *Phradis interstitialis* and *Tersilochus obscurator* (Hym.; Ichneumonidae) was a major factor for pollen beetle mortality in the soil. The mean level of parasitism increased from 47.5% in 1997 to 76.0% and 77.6% in 1998 and 1999, respectively. However, percent parasitism was not affected significantly by the tillage system applied before sowing of the oilseed rape crop. Consequently, the different emergence rates of new generation beetles from the different tillage systems may have been caused mainly by distinctive physical properties of the soil, regulatory effects of predators, or other factors.

Our results suggest that soil management practices before sowing of oilseed rape, like conservation tillage and no till, can be adequately integrated into the cropping system for reducing pollen beetle populations, thus contributing to Integrated Pest Management in oilseed rape. In other studies, reduced non-inversion tillage following harvest of oilseed rape has shown potential to increase the survival of pollen beetle parasitoids during hibernation in soil.

Key words: *Meligethes aeneus*, oilseed rape, tillage regimes, population dynamics, larval parasitism

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Role of architectural plasticity in the response of oilseed rape to flower pruning, simulating damage by pollen beetles (*Meligethes aeneus*)

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Abstract: Winter oilseed rape is a multipurpose crop (farm-produce industry for oil, cattle feed and biofuel) and is therefore becoming the focus of increasing interest in Europe and throughout the world. Moreover, winter oilseed rape is an excellent rotation crop for cereals as it helps to disrupt the cycle of soil-borne pathogens. However, winter oilseed rape suffers from a lot of insect pests (cabbage seed weevil, cabbage root fly, rape stem weevil, pollen beetle, etc.). Thus, current objectives for production of this crop are to maintain yield, seed quality and effective control of the pest populations.

The pollen beetle is responsible for important yield losses; beetles feed on pollen from flower buds or open flowers; the former leading finally to pod losses. The final yield losses will depend on both the pollen beetle attack intensity (which can be controlled at the landscape scale) and the compensation capacities of the crop plants. Our work focuses on the second strategy: improving compensation capacities of the plants. Many previous studies have investigated this topic, indicating that plant compensation capacities involve architectural plasticity. They show as an example that pruning induces (i) the development of vegetative and basal ramifications, (ii) the growth of pods that would otherwise have been aborted and (iii) the increase of seed weight. But the efficiency of these compensations is variable and conditions of significant compensation are still unknown.

Our hypothesises are (i) that the amount of reproductive organs (flowers and pods) that the plant can still produce after a pollen beetle attack is an important factor of plant's compensatory ability, (ii) genotypes with contrasting architectures and contrasting dynamics of flower development may have different compensation capacities.

To test these hypotheses we conducted an experiment in which we generated different dynamics of ramification and flower development. Therefore, we used two nitrogen (N) fertilisation treatments and three varieties with contrasting architectures (cv. Pollen, Gamin and Exocet). We assumed that pollen beetle attack could be simulated by flower pruning.

Plant compensation (i.e., the architectural plasticity) will be interpreted by the sink/source concept using a Structural Functional Plant Model. Our work will allow us to define architectural ideotypes that can better compensate for pollen beetle attack. The work will be carried out in the framework of a PhD studentship that started in September 2007. Our poster will present the main hypotheses and the results from the first year's experiments.

Key words: Brassica, yield losses, *Meligethes*, compensation, source/sink relationships, yield components, seed quality

Pathology papers

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Effect of sowing date on health status of open pollinated, composite hybrid and restored hybrid cultivars of winter oilseed rape (*Brassica napus* L.)

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Abstract: The aim of the study was to evaluate the effect of sowing date on the growth and development of the open pollinated ('Contact'), composite hybrid ('Kaszub') and restored hybrid ('Kronos') cultivars of winter oilseed rape. Higher degree of blackleg (*Leptosphaeria maculans, L. biglobosa*) and black spot (*Alternaria* spp.) symptoms were observed during the three years of investigations. Trace symptoms of infection with *Erysiphe cruciferarum, Sclerotinia sclerotiorum* and *Botrytis cinerea* were also noted. The occurrence of blackleg on winter oilseed rape was influenced by both experimental factors investigated. Significantly lower levels of infection of *Leptosphaeria* spp. were noted in optimal and late sown crops. Significantly, in each year of research, the cultivar with lowest levels of blackleg was 'Kaszub'. 'Kronos' the restored hybrid cultivar was significantly less susceptible to infection and the cultivar with the highest levels of blackleg was 'Contact', the open pollinated winter oilseed rape cultivar. There was no effect of the studied factors on black spot occurrence on any of the cultivars.

Key words: Oilseed rape, *Brassica napus*, blackleg, *Leptosphaeria*, black spot, *Alternaria*

Introduction

Winter oilseed rape (WOSR) is one of the most economically important plants cultivated in Poland. The interest in its cultivation increased systematically in recent years. Nowadays, farmers grow open pollinated, composite hybrid and restored hybrid WOSR cultivars. Hybrid cultivars are generally characterized by better growth and development characteristics, greater resistance to stress factors and higher yield compared to open pollinated cultivars. Because of these features, they can be more tolerant to a late sowing date.

WOSR can be attacked by numerous pathogens. Fungi of the genus *Leptosphaeria* spp. (*L. maculans* and *L. biglobosa*), are the casual agents of blackleg. Together with *Sclerotinia sclerotiorum*, and *Botryotinia fuckeliana* they are the most serious threats of WOSR in Polish climatic conditions (Sadowski *et al.*, 1995; 1998, Jedryczka *et al.*, 1999). They can strongly decrease yield. Another important oilseed rape disease is black spot caused by *Alternaria* spp. (Tewari, 1991). Fungi from these genera can also decrease yield quality due to the production of mycotoxins.

The aim of the study was to evaluate the effect of sowing date on plant growth and disease susceptibility of open pollinated, composite hybrid and restored hybrid cultivars of WOSR.

Material and methods

The experiment was conducted in 2003-2005 under field conditions in the Experimental Station in Balcyny (Department of Crop Production of University of Warmia and Mazury in Olsztyn). Contact (open pollinated), Kaszub (composite hybrid) and Kronos (restored hybrid) oilseed rape cultivars were used. The second factor was the sowing date which was varied as follows: the 12th of August (early), the 19th of August (optimal) and the 2nd of September (late). The field experiment was established using a split-plot design with four replications. Plot area was 15,8 m^2. Before sowing, the following doses of N, P, K were applied on all plots: 30 kg·ha^{-1}, 35 kg·ha^{-1}, 100 kg·ha^{-1}, respectively. The previous crop was spring barley. Soil conditions are explained in Table 1. Level of oilseed rape infection by pathogens was determined during the beginning of ripening. Fifty plants per plot were randomly chosen for evaluation. Percentage area of siliques with symptoms of infection with *Alternaria* spp. was estimated. Level of infection with *Leptosphaeria* spp. was evaluated using a 4 degree scale (0-3). Degrees of infection were transferred into a disease index (DI) (Wenzel 1948). Data were statistically analyzed using analysis of variance and means were compared using Tukey's test.

Table 1. Description of soil conditions

Specification	Vegetation period		
	2002/2003	2003/2004	2004/2005
Soil type	soil lessives typical		
Soil species	medium clay		
Soil pH (1 M KCl)	6.4	6.1	6.6
Soil valuation class	R-IIIa		
Soil suitability complex	good wheat		
Content of nutrients (mg kg^{-1} soil):			
— P	69.8	67.6	75.1
— K	120.4	141.1	139.5
— Mg	85	80	100

Results and discussion

Compared to other growing seasons, a high level of blackleg and black spot symptoms was observed during the three years of investigations (Tables 2 and 3). In contrast, trace levels of *Erysiphe cruciferarum, Sclerotinia sclerotiorum* and *Botrytis cinerea* were noted. Sadowski *et al.* (1995, 1998) observed that occurrence of the latter diseases, especially powdery mildew on a high level was rare under Polish climatic conditions. Blackleg was the disease occurring at highest severities during the years of investigation (Table 2). Many authors classify blackleg to be the most dangerous and the most prevalent disease of winter oilseed rape (Doughty *et al.*, 1995; Gladders & Symonds 1995; Sadowski *et al.*, 1998; Biddulph *et al.*, 1999). There was an influence of both studied factors on the occurrence of blackleg observed on winter oilseed rape plants. Significantly, lower levels of infection with *Leptosphaeria* spp. was noted in optimal and late sown plots. Early sown winter oilseed rape showed the highest

intensity of blackleg symptoms in each year of research. Significantly the lowest susceptible cultivar to blackleg infection in each year was ‘Kaszub’.

Table 2. Occurrence of blackleg (*Leptosphaeria maculans, L. biglobosa*) symptoms on winter oilseed rape cultivars (Balcyny 2003-2005). [Disease incidence in %]

Cultivar (A)	Sowing terms (B)			
	Early	Optimal	Late	Mean
2003				
Contact	23.3	11.0	3.8	12.7
Kaszub	16.8	5.5	5.0	9.1
Kronos	18.8	8.5	5.5	10.9
Mean	19.6	8.3	4.8	10.9
$LSD_{\alpha = 0.05}$: A – 1.97*, B – 1.97, B/A – 3.42, A/B – 3.42				
2004				
Contact	31.3	14.5	14.5	20.1
Kaszub	20.8	17.5	15.8	18.0
Kronos	23.0	18.0	19.3	20.1
Mean	25.0	16.7	16.5	19.4
$LSD_{\alpha = 0.05}$: A – 1.82, B – 1.82, B/A – 3.16, A/B – 3.16				
2005				
Contact	29.8	24.9	27.8	27.5
Kaszub	12.1	6.4	7.5	8.7
Kronos	9.6	12.3	11.5	11.1
Mean	17.2	14.5	15.6	15.8
$LSD_{\alpha = 0.05}$: A – 1.18, B – 1.18, B/A – 2.05, A/B – 2.05				
2003 - 2005				
Contact	28.1	16.8	15.3	20.1
Kaszub	16.5	9.8	9.4	11.9
Kronos	17.1	12.9	12.1	14.0
Mean	20.6	13.2	12.3	15.3
$LSD_{\alpha = 0.05}$: A – 1.15, B – 1.15, B/A – 1.99, A/B – 1.99				

‘Kronos’, the restored hybrid cultivar, was significantly lower susceptible to infection to blackleg in comparison to ‘Contact’ the open pollinated winter oilseed rape cultivar which was the most susceptible. Jankowski & Budzynski (2007) showed that this cultivar had the lowest level of yield compared to ‘Kronos’ and ‘Kaszub’. Similar results were obtained in COBORU (2007) research. The reason can be a lower resistance of ‘Contact’ to pathogens’ attack. Moreover, the content of nitrogen, sulphur, boron and magnesium in the soil can highly influence the cultivars’ resistance to diseases. Lack or low level of these macro and microelements can result in higher susceptibility to pathogens (Schnug & Ceynowa, 1990; Schnug *et al.*, 1995; Grzebisz & Gaj, 2000; Wielebski, 2000; Fabry *et al.*, 2000). The studied hybrid winter oilseed rape cultivars showed lower susceptibility to diseases than the open pollinated.

Table 3. Occurrence of black spot (*Alternaria* spp.) symptoms on winter oilseed rape cultivars (Balcyny 2003-2005). [Disease incidence in %]

Cultivar (A)	Sowing terms (B)			
	Early	Optimal	Late	Mean
2003				
Contact	2.5	3.0	4.5	3.3
Kaszub	2.3	2.5	3.0	2.6
Kronos	3.3	4.0	4.5	3.9
Mean	2.7	3.2	4.0	3.3
$LSD_{\alpha = 0.05}$: A – n.s.*, B – n.s., B/A – n.s., A/B – n.s.				
2004				
Contact	1.3	0.7	1.0	1.0
Kaszub	1.6	1.3	1.1	1.3
Kronos	0.9	0.8	1.1	0.9
Mean	1.3	0.9	1.0	1.1
$LSD_{\alpha = 0.05}$: A – n.s., B – n.s., B/A – n.s., A/B – n.s.				
2005				
Contact	0.5	0.4	0.5	0.5
Kaszub	0.5	0.6	0.4	0.5
Kronos	0.4	0.5	0.5	0.5
Mean	0.5	0.5	0.5	0.5
$LSD_{\alpha = 0.05}$: A – n.s., B – n.s., B/A – n.s., A/B – n.s.				
2003-2005				
Contact	1.5	1.4	2.0	1.6
Kaszub	1.4	1.4	1.5	1.5
Kronos	1.5	1.8	2.0	1.8
Mean	1.5	1.5	1.8	1.6
$LSD_{\alpha = 0.05}$: A – n.s., B – n.s., B/A – n.s., A/B – n.s.				

Severities of black spot symptoms were very low (Table 3). The highest level of infection with *Alternaria* spp. was observed in 2003 and did not exceed 4.5%. There was not any effect of studied factors on black spot occurrence on the plants, even though in some years black spot can be the main disease of winter oilseed rape (Songin *et al.*, 1989; Sadowski & Budzynski, 1995).

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Phenotypic distortions of winter rapeseed in successive generations after primary infection with phytoplasmas

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Abstract: Every year "shaggy" plants infected with phytoplasmas can be observed in winter rapeseed crops. The symptoms are so characteristic that finding such affected plants is very easy. At present it is possible to identify the pathogen and its polymorph forms by molecular methods (DNA analysis). Deformation of plants can occur before flowering or it can also appear during and after the flowering period. Seed development depends on the time when the symptoms begin to develop. In general seeds do not set when the changes are present before rapeseed plants blossom. In the case of late symptoms, a small amount of seeds can be expected and these are capable of germination. The flowers of infected plants are strongly deformed. Petals usually turn into leaf-like, light-green forms with empty, distended 5cm long pistils. Using DNA-analysis, our survey confirmed that the reason of the disease was undoubtedly the presence of phytoplasmas. Similar symptoms on rapeseed plants have been described earlier, but analysis of the progeny of infected plants was not performed. Analysis of progeny raised from homozygotic forms (DH lines) was performed in the Laboratory of Resistance Breeding Method of PBI (IHAR) in Poznań. Seeds were collected from isolated plants which had visible symptoms of phytoplasma disease. Isolation of flowers with paper isolators allowed self-pollination and the next generation comprised of deformed plants. The deformations caused strange shape of plants such as "creeping" or funnel-like leaves. The plants were used in crossing programs. It was found that distortions have fixed character and they have hereditary character. In all androgenic, haploid and double-haploid plants grown *in vitro*, phenotypic deformations were observed. Some fragments of primary distorted plants were grown *in vitro* conditions on agar medium (Gamborg B_5) with antibiotics (carbenicillin). Stems which were cut off from treated plants were healthy. After re-planting in soil they developed as normal rapeseed plants and produced normal seeds and the plants made a full recovery. In contrast, the offspring of infected plants showed fixed deformations which suggests that the changes caused by phytoplasmas are at the DNA level (mutations) and there was therefore no possibilities to recover these mutated plants.

The results provide valuable information for breeders and seed producers and indicate the need for deformed shaggy-like infected plants to be removed from the breeding fields, nurseries and seed plantations because their pollen can be a source of infection for future generations.

Key words: oilseed rape, phytoplasma

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Pathogenic mycobiota of rape in Belarus

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Abstract: In the article the specific composition of winter and spring rape fungal diseases in Belarus was presented. 16 fungal species: *Alternaria brassicae* Sacc, *A. brassicicola* Wilts, *A. alternata* (Fr.) Keissler; *Sclerotinia sclerotiorum* (Lib.) de Bary; *Sclerotinia trifoliorum* Eriksson; *Fusarium oxysporum* f. sp. *brassicae* (Schlecht.) Snyd. et Hans.; *Fusarium avenaceum* (Fr.) Sacc.; *Fusarium nivale* (Fr.); *Typhula incarnata* Jasch. ex Fr.; *Erysiphe cruciferarum* Oxiz. et Junell; *Phoma lingam* (Tode) Desm.; *Cylindrosporium concentricum* Grev.; *Botrytis cinerea* (Fr.); *Olpidium brassicae* (Wor.) Dang; *Pythium* spp. were revealed and identified.

The symptomatology and forms of disease manifestation were described. Their spread and harmfulness was determined. The most economically important oilseed rape diseases in Belarus were identified as *Alternaria* blight, *Sclerotinia* stem rot, *Fusarium* wilt and gray mould.

Introduction

In Belarus, oilseed rape has been grown for more than 20 years and is a major oil crop. At present cultivation represents more than 2% of the crop-sown areas of the Republic and the area continues to increase annually. Breeding and the rational introduction of local rape varieties adapted to specific soil-climatic conditions of districts of the Republic has increased rape field productivity. However, the regionalized varieties of local and foreign selection and also varieties and variety samples in field tests are not resistant to diseases infecting winter and spring rape. In addition, increased cropping of mustard family crops has added to phytosanitary concerns for oilseed rape growers.

Until now, oilseed rape diseases have not been studied and reported in Belarus.

Material and methods

Monitorings of oilseed rape crops was carried out by undertaking regular inspections and samples of infected plants were analysed subjected to microbiological analysis. Pathogen identification was based on disease symptoms and spore morphology.

Results

Phytopathological inspections of winter and spring rape indicated a specific composition of mycobiota on oilseed rape across Belarus data on specific pathogens and the infected oilseed rape organs identified are presented in the Table 1.

Table 1. Fungal diseases and diseased organs of winter and spring oilseed rape observed in Belarus

Disease name	Agent	Disease symptom / pathogen on				
		Roots	Stems	Leafs	Pods	Seeds
Black leg	*Olpidium brassicae* (Wor.) Dang; *Pythium* spp.	+	-	-	-	-
Alternaria blight	*Alternaria brassicae* Sacc; *A. brassicicola* Wilts; *A. alternata* (Fr.) Keissler	+	+	+	+	+
White rot	*Sclerotinia sclerotiorum* (Lb.) de Bariy;	+	+	+	+	-
Fungal wilt	*Fusarium oxysporum* f. sp. *brassicae* (Schlecht.) Snyd. et Hans.	+	+	-	-	+
Peronosporosis (downey mildew)	*Peronospora brassicae* Gaeumann	-	+	+	+	+
Powdery mildew	*Erysiphe cruciferarum* Oxiz. et Junell	-	+	+	+	-
Phomosis (Stem Canker)	*Phoma lingam* (Tode) Desm. Ascomycetous stage – *Leptosphaeria maculans* Ceset (Desm) pes et De Not.	+	+	+	+	+
Cylindrosporium disease (Light leaf spot)	*Cylindrosporium concentricum* Grev. Ascomycetous stage – *Pyrenopeziza brassicae*	-	+	+	+	-
Gray mould	*Botrytis cinerea* (Fr.) Ascomycetous stage – *Botryotinia fuckeliana* (D.B.) Whet	+	+	+	+	+
Snow mould	*Fusarium nivale* (Fr.) Ces.;	+	+	+	-	-
	Sclerotinia trifoliorum Eriksson	+	+	+	-	-
	Typhula incarnata Jasch. Ex Fr.	+	+	+	-	-
Root rots	*Fusarium avenaceum* (Fr.) Sacc.	+	+	-	-	-

***Alternaria* blight and black spot** are found everywhere in Belarus in winter and spring rape, the spread and development of which is determined by weather conditions during vegetation. In dry seasons such as 1999 and 2002 the amount of precipitation during the whole vegetation period was 35-75% lower than the long-term mean whilst accumulated degree-day temperature exceeded the long-term mean by between 250-450 °C. *Alternaria* blight development in oilseed rape was of a reduced and did not increase above 20.5%. In 2000-2004 (except 2002) there was an epidemic of *Alternaria* blight with the disease levels ranging between 74.4-80.5% (precipitation level was 1-1.5 times the long-range mean whilst the average air temperature fluctuated within the limits of a long-range mean. In Belarus *Alternaria* blight was found to be caused by *Alternaria brassicae* Sacc, *A. brassicicola* Wilts,

and *A. alternata* (Fr.) Keissler. The disease symptoms on oilseed rape with the enumerated pathogen species show some differences.

In the cases of *Alternaria brassicae* Sacc. infection of oilseed rape plants, cotyledon leaves developed dark-brown spots which caused rotting and hypocotyl death during the early stages of ontogenesis. On true leaves, light-smoke coloured spots with a light halo round the spot were formed. Sporulation produced dark concentric rings up to 1 cm in a diameter from the center. On infected pods dark, round, depressed infections start to appear causing pod deformation. The stems are covered by oblong dark spots.

A. brassicicola Wilts. caused rotting of the radicle during seed germination. On leaves, light-brown spots of arbitrary form without rings were formed. On pods, dark spots and necrotic infections were seen and the pod tip darkened. On stems dark spots were observed. With infections caused by *A. alternata* (Fr.) Keissler again, the pod tip appeared to die off and this spread to the whole organ which was covered with abundant dark spores. On leaves and stems dark spots formed. Infection by this species was marked in years of moisture deficiency or when oilseed rape plants are weakened due to lack of nutrition. In years of epidemic years pod length was decreased by 8-26%, seed number was decreased for 12-59%, 1000 seed weight reduced by 15-70% and seed oil content was reduced by 11-27%. The infection of *Alternaria* blight continued from crop to crop in the form of mycelium and conidium on infected winter oilseed rape leaves, on plant residues of mustard crops and seeds. Infection of oilseed rape seed by fungi genus *Alternaria* makes 37-90% of total seed infections.

Sclerotineose (white rot) was found universally throughout winter and spring oilseed rape crops in Belarus, *Sclerotinia* infection was observed on rots, stem, leaves and pods. However, during the current study, we did not identify resistant or badly infected winter and spring rape varieties in Belarus.

Other diseases identified were grey mould (*Botrytis cinerea* (Fr.), *Fusarium* wilt. *Fusarium* spp., downy mildew (peronosporosis) *Peronospora brassicae* Gaeumann, powdery mildew (*Erysiphe cruciferarum* Oxiz. et Junell), Phomosis (*Leptosphaeria maculans* Ceset (Desm) pes et De Not), light leaf spot (cylindrosporium disease) (*Pyrenopeziza brassicae*)**,** and blackleg (*Olpidium brassicae* (Wor.) Dang; *Pythium* spp.).

First occurrence of ring spot (*Mycosphaerella brassicicola*) in France

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Abstract: For two years ring spot like symptoms have been observed in the west coast region of France. On leaves, lesions were round, light brown to grey-brown, bearing numerous small black fruiting bodies and surrounded by a yellow halo. They could be confused with *Phoma* leaf spot and *Alternaria* leaf spot. Later in the season, similar symptoms were observed on pods. Although the fungus could not be isolated, the causal agent could be *Mycosphaerella brassicicola.* The appearance of the new epidemics could be due to high humidity and mild temperatures during the winter. Some DMI fungicides sprayed at stage 60 were efficient but did not provide control on pods before harvest.

Key words: winter oilseed rape, ring spot, *Mycosphaerella brassicicola*, chemical control

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Potential impact of a changing climate on *Phoma* stem canker and light leaf spot of oilseed rape in the UK

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Abstract: *Phoma* stem canker (*Leptosphaeria maculans*) and light leaf spot (*Pyrenopeziza brassicae*) are the two most serious diseases of winter oilseed rape in the UK. Despite expenditure of more than £20M on fungicides each growing season, these two major diseases account for more than £120M of losses (at a price of £225 t^{-1}). The distribution of each disease is affected by climate, with *Phoma* stem canker most severe in the warmer, drier south and east of the UK and light leaf spot most severe in the wetter, cooler west and north with epidemics being particularly severe in Scotland. Little work has been done to predict the impacts of climate change on plant disease epidemics. To investigate possible impacts, a weather-based disease forecasting model for *Phoma* stem canker was combined with a climate change model predicting UK temperature and rainfall under high and low CO_2 emissions for the 2020s and 2050s. Multi-site data collected over a 15-year period from across the UK were used to develop and validate the model to forecast the severity of epidemics on oilseed rape. The model predicted that *Phoma* stem canker epidemics will increase in severity and the range of the disease will spread northwards into Scotland by the 2020s. However, using the same climate change scenarios, a weather-based light leaf spot forecast model predicted that light leaf spot will become less serious throughout the UK, especially in southern England.

Crop protection and resistance to these two major UK pathogens make important contributions to climate change mitigation, since low-yielding diseased crops use more nitrogen fertilizer per ton of grain and require more crop-area to achieve the same national yield of oilseed rape. This work suggests that predictions of impacts of climate change on other plant diseases are needed to guide policy and practice in adapting to impacts of climate change on food security, environment and wildlife.

Key words: Food security, global warming, light leaf spot, *Phoma* stem canker, sustainability

Introduction

Current estimates suggest that globally more than 1 billion people do not have sufficient food (Anon., 2009). As the world's population continues to increase, the situation will continue to worsen since crop losses from diseases are estimated at 16% globally, despite efforts to control them (Oerke, 2006). The food security problems associated with crop diseases are now becoming more acute due to climate change (Anderson *et al*., 2004; Chakraborty *et al*., 2000; Garrett *et al*., 2006; Gregory *et al*., 2009; Stern, 2007).

Oilseed rape (*Brassica napus*) is grown throughout the world as a source of oil and protein (for human/animal consumption) and fuel (e.g. as a component of biodiesel). A disease of global importance on oilseed rape is *Phoma* stem canker (blackleg, caused by *Leptosphaeria maculans*), which results in losses amounting to more than £500M per season through severe epidemics in Europe, North America and Australia, and is spreading globally, threatening production in India, China and Africa (Fitt *et al*., 2006; Fitt *et al*., 2008). Another disease of importance in northern Europe is light leaf spot (caused by *Pyrenopeziza brassicae*) (Boys *et al*., 2007). These are the two most important diseases of oilseed production in the

UK, where yields are generally >3 t ha^{-1}, with *Phoma* stem canker currently being more important in southern England and light leaf spot being more important in northern England and Scotland (www.cropmonitor.co.uk). This paper uses a combination of predicted climate change data and disease risk prediction models to examine the potential impact of climate change on the two main oilseed rape diseases of winter oilseed rape in the UK. Experiments were also done to investigate the effect of increased temperature on major gene mediated resistance to *L. maculans* the *Phoma* stem canker pathogen.

Material and methods

Climate change scenarios and oilseed rape disease predictions

Daily site-specific climate scenarios were generated as described in Evans *et al*. (2008). There were five simulated climate scenarios; baseline (1960-1990) and 2020HI, 2050HI, 2020LO and 2050LO for high and low CO_2 emissions for the 2020s and 2050s. Daily weather data for 30 years were generated for the five climate scenarios by a stochastic weather generator (LARS-WG, Semenov and Barrow, 1997) for 14 sites across the UK. Data generated were daily minimum temperature, maximum temperature and rainfall. These weather data for different climate scenarios were used as the inputs into weather-based models for predicting the severity of *Phoma* stem canker disease (Evans *et al*., 2008) and the incidence of light leaf spot disease (Welham *et al*., 2004).

Controlled environment experiments with ascospore inoculum and GFP-expressing transformed isolates of L. maculans

Plants of DarmorMX (with resistance gene *Rlm6*) and Darmor were grown in pots (5 cm diameter) containing peat-based compost and a soluble fertiliser. Plants were initially grown in a glasshouse (20-23 °C) with one plant per pot and placed in seed trays (37 cm × 23 cm) with 14 plants (seven plants of Darmor and seven plants of DarmorMX) per tray. Three weeks after sowing, the plants were transferred to a 15 °C controlled environment cabinet (12 h light: 12 h darkness, light density 210 µe m^{-2} s^{-1}) until plants had three expanded leaves and was ready for inoculation. Plants were inoculated and scored as detailed in Huang *et al*. (2006).

Results and discussion

Predicted impact of climate change on severity of Phoma stem canker and incidence of light leaf spot

The predicted severities of phoma stem canker at harvest for the 2020s and 2050s were much greater than during 1960-1990; the UK maximum mean severity increased from 1.7 (1960-1990) to 2.0 (2020s) and 2.3 (2050s) on the 0-4 scale for a harvest date of 15 July (Figure 1). These increases in severity of epidemics still occurred if harvest dates were earlier, as predicted for sites in southern England, when harvest dates under these scenarios were estimated for Rothamsted from predicted wheat harvest dates (Evans *et al*., 2008).

In contrast with the predicted increase in range and severity of *Phoma* stem canker epidemics, the incidence of light leaf spot was predicted to decrease (Figure 2). For example, in northern England and Scotland, the incidence of light leaf spot is predicted to decrease by 20-30% plants affected under predicted climate change scenarios for the 2050s (Figures 2 d &

e). In the south of England, where the incidence of severe light leaf spot epidemics is currently small, it is predicted to decrease further.

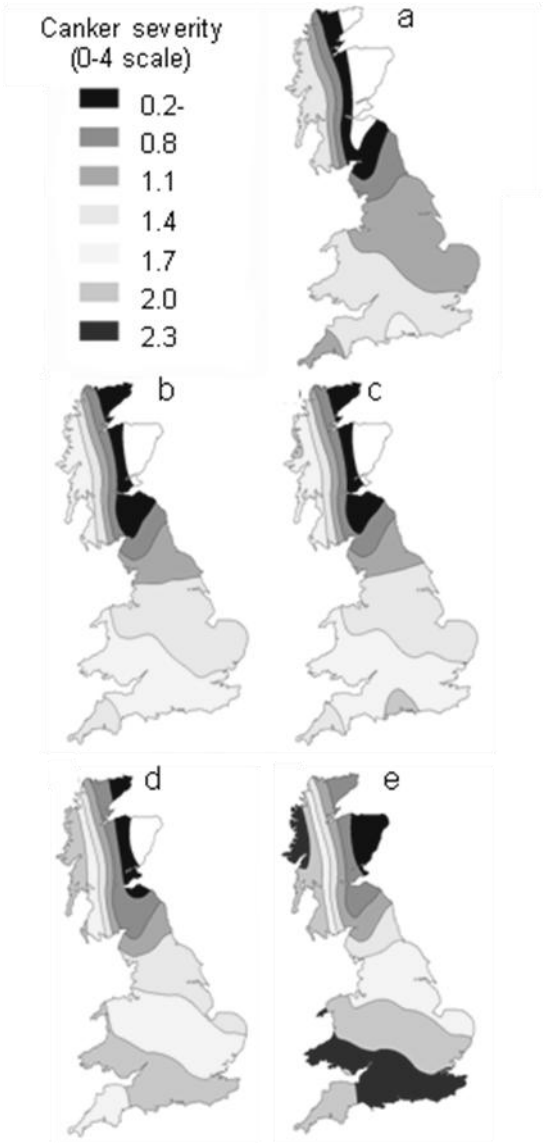

Figure 1. Predicted severity of *Phoma* stem canker (*Leptosphaeria maculans*) at harvest on winter oilseed rape crops (cultivars with average disease resistance) for (a) baseline 1960-1990, (b) 2020s low emissions, (c) 2020s high emissions, (d) 2050s low emissions and (e) 2050s high emissions climate scenarios. Stem canker severity on a 0-4 scale (0, no disease; 4, plant dead; Zhou *et al*., 1999). Areas unaffected by the disease are white. Predicted severities are interpolated from predictions for 14 sites across the UK.

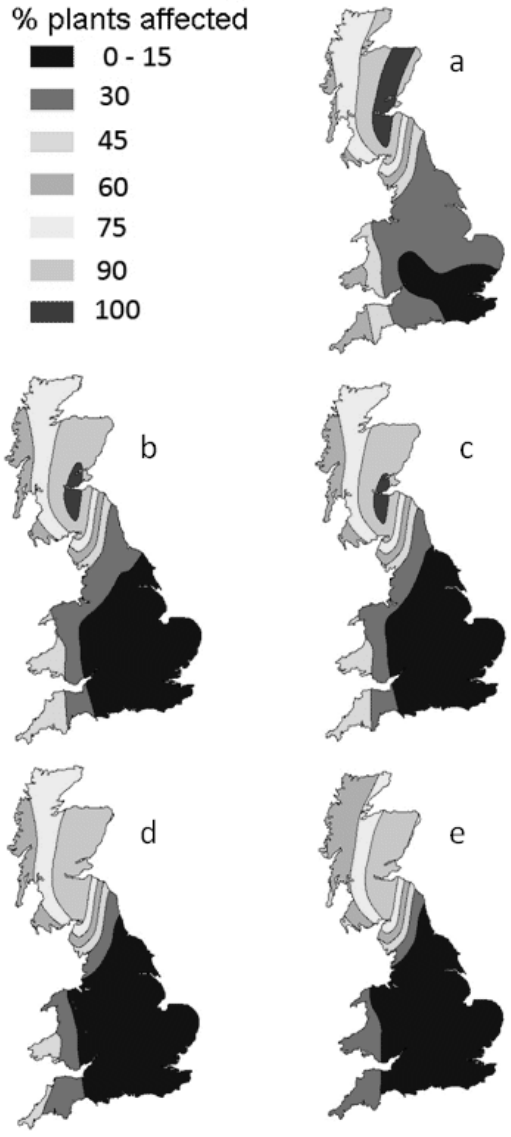

Figure 2. Predicted incidence (% plants affected) of light leaf spot (*Pyrenopeziza brassicae*) at green flower bud (GS 3,3) of UK winter oilseed rape crops (cultivars with average disease resistance) for (a) baseline 1960-1990, (b) 2020s low emissions, (c) 2020s high emissions, (d) 2050s low emissions and (e) 2050s high emissions climate scenarios. Predicted incidences are interpolated from predictions for 14 sites across the UK.

Phoma leaf spot phenotype

In experiments with the ascospore inoculum, disease symptoms differed between DarmorMX (carrying *Rlm6*) and Darmor (lacking *Rlm6*) at temperatures of 5-20 °C. On Darmor for all temperature and wetness duration treatments, large grey lesions (> 2 mm in diameter; Figure 3a; typical grey *Phoma* leaf lesions) had developed by 5 (25 °C) to 18 d (5 °C) after inoculation. On DarmorMX, the symptoms differed between temperatures. At 5-10 °C, no visible symptoms were observed up to 18 d after inoculation, when the leaves were still green (as in Figure 3b). However, a few small dark spots (< 2 mm in diameter; as in Figure 3d, typical small dark spots) or 'green islands' (i.e. leaf areas around sites of successful penetration with delayed senescence in comparison with the rest of the leaf tissue; as in Figure 3c) were observed on some leaves 21-30 d after inoculation, when the leaves had turned yellow and senesced. At 15 °C, by 11 d after inoculation no visible symptoms had developed on DarmorMX (Figure 3b), whereas large grey lesions had developed on Darmor (Figure 3a). Small dark necrotic spots were not observed on DarmorMX at 15 °C until 14 d after inoculation. At 20 °C, small dark necrotic spots (Figure 3d) were observed on DarmorMX 8 d after inoculation, when the leaves were green. At both 15 and 20 °C, by 18-23 d after inoculation when the leaves had senesced, the small dark spots remained small (< 2 mm in diameter) and 'green islands' were also observed (Figure 3c). At 25 °C, large grey lesions developed on DarmorMX (Figure 3e) by 6 d after inoculation. *L. maculans* isolates were obtained from both small dark spots and 'green islands' which developed on DarmorMX at 5-20 °C. In experiments with conidial inoculum, at 15 °C large grey lesions developed on Darmor 14 d after inoculation, but very few visible symptoms developed on DarmorMX, even when the leaves senesced; however, *L. maculans* was re-isolated from the inoculated sites on symptomless leaves. At 25 °C, large grey lesions developed both on Darmor (Figure 3f) and DarmorMX (Figure 3g) by 10 d after inoculation.

Systemic growth from leaf lesions towards stems

On leaves of DarmorMX inoculated with the conidia of GFP-expressing *L. maculans* at 15 °C, localised cell necrosis around the inoculation site was observed. Growth of *L. maculans* was confined within these small necrotic areas (Figure 4a). Necrosis was not observed on DarmorMX at 25 °C (Figure 4d) or on Darmor at either 15 °C or 25 °C (Figure 4b). Whereas on DarmorMX small dark spots with no pycnidia were produced at 15 °C (Figure 4a), large lesions with pycnidia developed on DarmorMX at 25 °C (Figure 4d). On Darmor at both 15 °C and 25 °C, large lesions with pycnidia were produced (Figure 4b). On Darmor at 15 °C and 25 °C and on DarmorMX at 25 °C, hyphae of GFP-expressing *L. maculans* grew down the leaf petiole towards the stem (Figure 4c). These hyphae growing down the petiole towards the stem were mainly travelling through xylem vessels or between cells of the xylem parenchyma and cortex (Figure 4c). At 15 °C, 36 d after inoculation, after the inoculated leaves had dropped off, hyphae of GFP-expressing *L. maculans* were observed on leaf scars of Darmor (Figure 4f) but not on DarmorMX (Figure 4h). However, at 25 °C, 31 d after inoculation, after the inoculated leaves dropped off, GFP-expressing hyphae were observed on leaf scars of both Darmor (Figure 4j) and DarmorMX (Figure 4l).

Conclusions

In the UK, it is predicted that climate change will increase the severity of epidemics caused by *Phoma* stem canker, which is favoured by increased temperature (Evans *et al*., 2008) but decrease the severity of epidemics caused by light leaf spot, which is adversely affected by

the predicted higher temperatures (Boys *et al*., 2007). These contrasting impacts of climate change on different diseases emphasise the need for detailed assessments of the impacts of climate change on specific diseases. However, early assessments of such impacts were frequently based on qualitative reasoning that could not accommodate the complex host-pathogen-environment interactions involved (Anderson *et al*., 2004). Analysis of specific plant-pathogen interactions (in this case *L. maculans* and *B. napus* lines with or without *Rlm6*) indicated that environmental factors may affect interactions between *L. maculans* effector genes and *B. napus* resistance genes. The influence of temperature on the effectiveness of *Rlm6* against *L. maculans* may help to explain why *Phoma* stem canker is most severe on oilseed rape in Australia, where temperatures during the growing season are higher than those in northern Europe. This work suggests that, should there be a rapid climate change with increasing temperature in Europe, there is the potential for more severe *Phoma* stem canker epidemics.

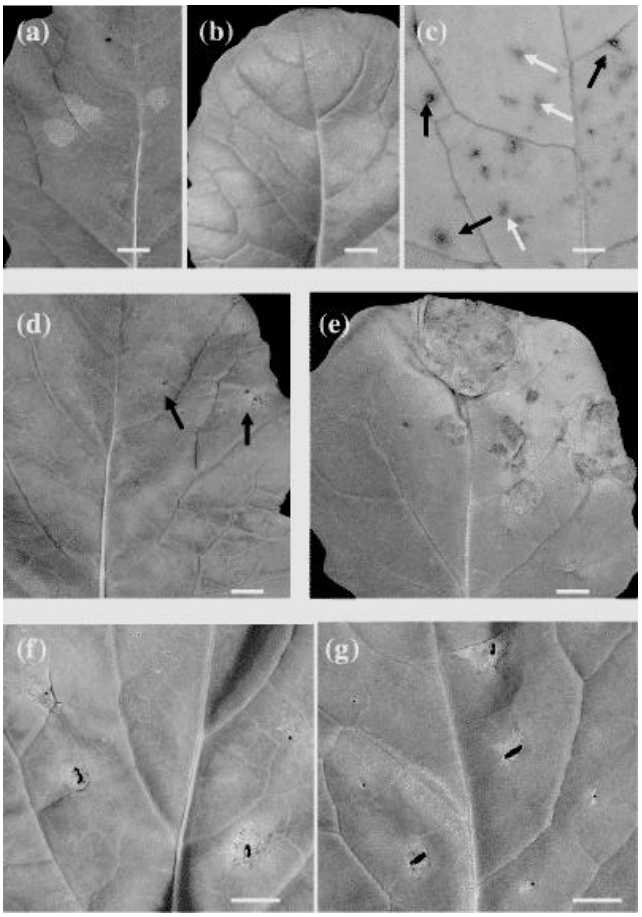

Figure 3. Symptoms on the leaves of *Brassica napus* Darmor (lacking *Rlm6*) and DarmorMX (carrying *Rlm6*) inoculated with ascospores (without wounding, a,b,c,d,e; with 48 h wetness) or conidia (after wounding, f,g; with 72 h wetness) of *Leptosphaeria maculans* carrying the effector gene *AvrLm6*. (a) Large grey lesions on Darmor 11 d after inoculation at 15 °C; (b) no visible symptoms on DarmorMX 11 d after inoculation at 15 °C; (c) small dark spots (black arrows) and green islands (white arrows) on DarmorMX 18 d after inoculation at 15°C; (d) small dark spots (arrows) on DarmorMX 11 d after inoculation at 20 °C; (e) large grey lesions on DarmorMX 16 d after inoculation at 25 °C; (f) large grey lesions on Darmor 16 d after inoculation at 25 °C; (g) large grey lesions on DarmorMX 16 d after inoculation at 25 °C. Bar, 5 mm.

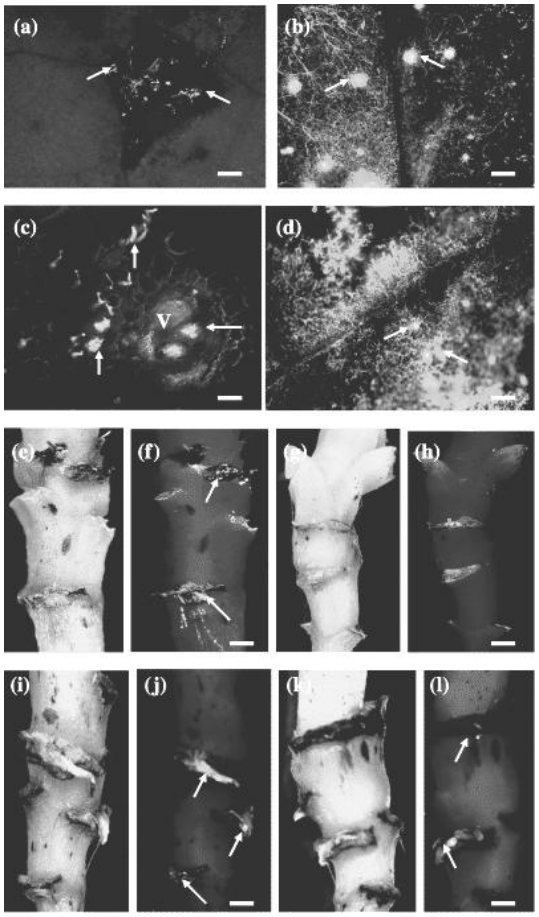

Figure 4. Development of *Leptosphaeria maculans* in leaves and stems of *Brassica napus* DarmorMX (carrying *Rlm6*) or Darmor (lacking *Rlm6*), demonstrated by the inoculation of leaves with conidia of an *L. maculans* isolate (carrying *AvrLm6*) expressing green fluorescent protein (GFP) at 15 °C or 25 °C, viewed under fluorescence (a-d,f,h,j,l) or white light (e,g,i,k). (a) necrotic response on leaves of DarmorMX at 15 °C, GFP *L. maculans* (arrows) associated with dead plant cells (brown) but not healthy plant cells (red auto-fluorescence); (b) hyphae and pycnidia (arrows) of GFP *L. maculans* within a leaf lesion on Darmor at 15 °C; (c) leaf petiole cross-section of Darmor, showing colonisation of vascular bundle (V) and surrounding tissue at 25 °C; (d) hyphae and pycnidia (arrows) within a leaf lesion on DarmorMX at 25 °C. *L. maculans* had spread down petioles to reach stems of Darmor (e,f) but not DarmorMX (g,h) at leaf scars (arrows) by 36 d after inoculation at 15 °C; *L. maculans* had spread down petioles to reach stems of both Darmor (i,j) and DarmorMX (k,l) at leaf scars (arrows) by 31 d after inoculation at 25 °C. Bar, 200 µm (a,b,d), 100 µm (c) or 1 mm (e-l).

Acknowledgements

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Detecting airborne inoculum to forecast oilseed rape diseases

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Abstract: Many fungal diseases of oilseed rape are caused by air-dispersed spores. The timing of spore release changes each season due to the weather because the maturation and release of spores is usually affected by moisture (rain, dew and relative humidity) and temperature. Most airborne spores are dispersed only short distances from a source but many others still travel long distances, although spore concentrations and viability in air reduces over time. Spore deposition results in individual disease foci when occurrence of viable inoculum and infection conditions are rare; as a gradient in a crop when a large number of spores are produced from a nearby source; or as a uniform infection when there is a large but distant source or multiple local sources of inoculum. Epidemics of common monocyclic diseases of widespread crops, such as *Phoma* stem canker and *Sclerotinia* stem rot of oilseed rape are usually initiated by airborne spores produced either a long distance from the crop or from multiple sites throughout a region. Therefore it should be possible to predict such epidemics regionally using suitably located spore samplers in order to enhance integrated disease control methods. Appropriate DNA-based diagnostic methods can be integrated with many different types of air samplers and are now providing new information about species that previously could not be identified accurately by visual microscopy methods. For example, a new diagnostic for *Sclerotinia sclerotiorum* (Rogers *et al*., 2009) has shown potential for warning of the presence of airborne inoculum. Furthermore, where reliable climate-based disease forecasts have been developed (e.g for *Leptosphaeria maculans*), air sampling integrated with DNA-based diagnostics can also provide useful information at the sub-species level, to monitor populations for traits such as the development of fungicide resistance (in a similar way to that found with strobilurin resistance in *Mycosphaerella graminicola* (Fraaije *et al*., 2005) or changes to the pathogen race-structure in response to deployment of resistant cultivars. The optimal location of air samplers depends on how widespread the host crop is and how common the pathogen is, since air samples, particularly at ground level are heavily weighted in favour of spore types produced nearby. Further work is required to investigate the spatial variability in spore numbers in air at different sites; how changes in numbers of airborne spores at particular heights above or distances away from crops are related to subsequent regional disease severity; and to develop methods to analyse samples and disseminate results rapidly.

Key words: qPCR, air sampling, airborne spores

Introduction

Many fungal diseases of oilseed rape are initiated and spread by airborne spores. The timing of spore release varies from year to year because different weather conditions affect spore development and release. Disease often occurs several days after spores are first trapped by air-sampling techniques, when the disease incubation period has been completed (Figure 1a). This allows time for integrated crop protection measures to be applied with full efficacy. Furthermore, a number of studies have shown a correlation between airborne spore numbers and subsequent disease incidence or severity (Figure 1b). Thus in additon to improving knowledge of disease epidemics retrospectively, air sampling can potentially be used to

provide a timely inoculum-based disease warning, especially if it is linked to information about infection conditions.

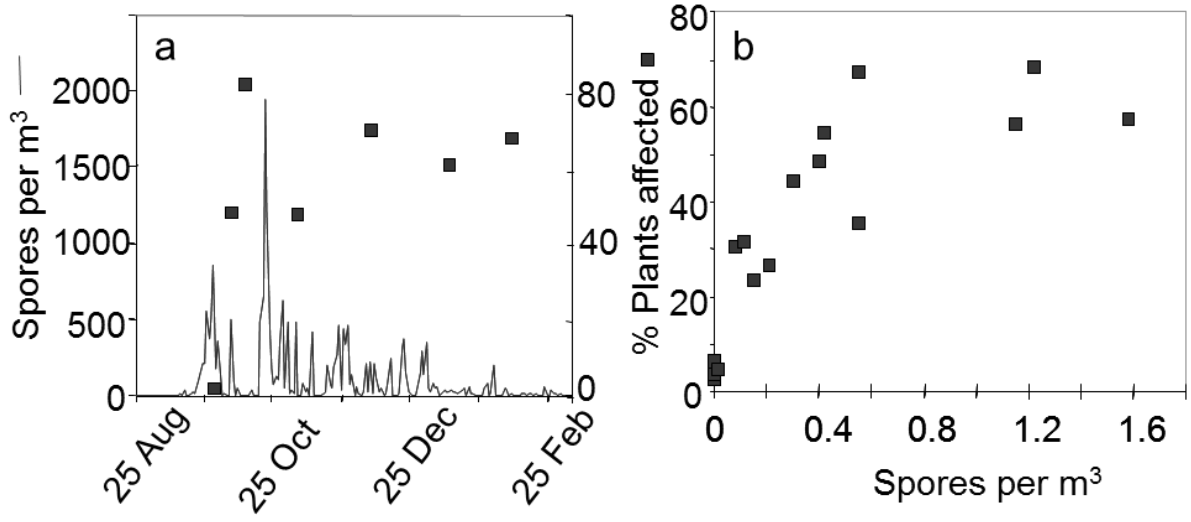

Figure 1. Example of the delay between first spores trapped (ascospores of *Leptosphaeria maculans*) and disease (*Phoma* leaf spot) (a); Relationship between the concentration of *Sclerotinia sclerotiorum* ascospores trapped in air above oilseed rape plots using rotating-arm samplers and the subsequent development of *Sclerotinia* stem rot several weeks later (b). Disease incidence (% plants affected) was linearly related to spore numbers up to a threshold at which other factors are thought to have limited disease development.

The two main air samplers used in plant pathology studies until recently are the Burkard seven day continuously recording sampler (based on the Hirst spore trap), which samples at 10 l air per min, and the rotating-arm sampler (‘rotorod’), which samples at ≈150 l air per min. Traditionally spores sampled by these devices were identified and counted by microscopy and this method can be relatively quick for large and easily identified spores and has been used to provide data for the subsequent development of climate-based disease forecasting schemes (e.g. *Phoma* leaf spot, http://www.rothamsted.ac.uk/Content.php?Section=Leafspot). However, visual identification and counting of spores by microscopy is potentially insensitive because only a small proportion of the tape is usually examined. It is also often very time-consuming and not appropriate for many species because most spores cannot be identified visually to the species level. Visual identification can be improved by using specific fluorescent antibodies (with fluorescence microscopy) but it is often difficult to get specific antibodies for fungal species. Where this has been achieved, e.g. for *Mycosphaerella brassicicola* and *Botrytis cinerea*, the microtitre immuno spore trap, MTIST, has been developed to enable rapid quantification of spores by ELISA (Kennedy *et al.*, 2000). Further developments such as lateral flow devices are enabling immunological methods to be used for rapid, on-site detection of inoculum (Kennedy *et al*., 2008).

Molecular methods

Integration of air sampling with DNA-based diagnostic techniques can provide a rapid and accurate detection and quantification of spores for species that are difficult to identify visually. Furthermore, with the correct markers, this method can provide genetic information about the pathogen population, such as presence of toxin-production genes, fungicide resistance and even avirulence alleles. DNA-based diagnostics can be integrated with all types of air sampler currently used (West *et al*., 2008). Since airborne spores represent an unbiassed sampling method to study populations occurring on different cultivars, wild and crop plants and different geographical locations, and because it is possible to study genetic traits using molecular diagnostics, the method is therefore appropriate for studying changes in pathogen populations in response to selection pressures. A disadvantage of DNA-based diagnostics is that there is no destinction between viable and dead spores so this could lead to false warnings of disease if inoculum were dead. Further research is needed to establish how frequently false positives are likely to occur for different target pathogens. If it is a problem, a potential solution might be to base detection on the more transient RNA in each cell.

Recently many studies using molecular diagnostics and air sampling have used quantiative PCR to detect one or a few target organisms or genetic traits (e.g. Fraaije *et al.*, 2005). This is providing new information about the epidemiology of various diseases, such as sclerotinia stem rot (Rogers *et al*., 2009). Already microarrays are being used to detect numerous airborne particles in one test e.g. bacteria in aerosols (Brodie *et al.*, 2007) and a range of microbes (DNA Multiscan; http://www.labservices.uoguelph.ca/units/pdc/). However, further developments in biosensors and DNA-based detection methods are needed to enable rapid and on-site detection of a range of plant pathogens.

Interpreting results

One problem with the approach of using airborne inoculum to initiate disease warnings is that it is difficult to establish set thresholds of pathogen DNA in air that indicate a risk to crops. This is because the distance from the inoculum source to the air sampler is not known. Therefore it is not clear whether the spores are a concentrated 'cloud' that is about to disperse and dilute to a very low concentration over the region, or whether they have come from a distant source and thus are at that concentration throughout the region. The wind speed also affects the concentration of spores in air relative to their deposition or impaction onto plants. At high wind speeds, the crop exposure to inoculum can still be high if the concentration of spores in air is low. This problem is common to other aerobiological studies. The UK National Pollen and Aerobiology Research Unit (NPARU, http://pollenuk.worc.ac.uk/), which coordinates a network of Burkard air samplers to provide hayfever advice, base decisions on a proportional increase in numbers of particles above a background level. For many pathogens of oilseed rape, particularly monocyclic pathogens such as *L. maculans* and *S. sclerotiorum*, the timing of significant increases in spore numbers in air from a low or absent 'base-line' level is extremely important information for disease control, even if the actual crop exposure to spore numbers is not quantified directly.

Another consideration is that spore numbers in air are heavily weighted in favour of those produced close to the air sampler making the measurement. The best location of air samplers will therefore depend on how widespread a crop is and how common a pathogen is. Detection of a potentially devastating but rare pathogen of a rare crop will probably best be done by sampling air above that crop since this will relate to crop exposure and is more likely

to detect any secondary inoculum (if airborne) arising from a rare primary infection, compared to detecting air away from the specific crop area. Conversely, for more common arable crop pathogens, in theory, if a spore trap located away from arable fields, such as on the roof of a town-centre building, detected the presence of spores, it could be assumed that those spores were present in air in relatively large numbers in the region.

Over larger scales, networks of spore traps in different locations are being used to improve control of *Phoma* stem canker of oilseed rape in Poland (http:// cropnet.pl/dbases/spec) and blue mould (*Peronospora tabacina*) of tobacco (http:// www.ces.ncsu.edu /depts/pp/bluemold/) and soybean rust (*Phakopsora pachyrhizi*) in the USA (Isard *et al*., 2007). The bluemold website uses forward-tracking of airflow to identify areas at risk of receiving inoculum from a known source. Back-tracking can also be used to identify risk of air arriving at a point having passed a known inoculum/disease source.

Summary

It is now possible to process air samples for molecular diagnostic techniques (qPCR), providing accurate detection and quantification of DNA of different target species. This can provide both a disease warning and, with the appropriate genetic markers, detailed information that can be obtained or analysed retrospectively, such as changes in the genetic structure of pathogen populations, e.g. the extent of fungicide resistance or selection of pathotypes. Further research is needed to understand the variability of air sample data over local and regional scales and to develop methods to deliver results quickly to make practical monitoring programmes effective.

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Impact of cabbage root fly, *Delia radicum*, on infections of oilseed rape by *Verticillium longisporum* and *Phoma lingam*

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Abstract: Oilseed rape is an economically important crop in German Agriculture. As a result of intensive cultivation, an increasing incidence of some brassica-related fungal diseases and pests has been observed recently. *Verticillium longisporum* and *Phoma lingam* are causal agents of *Verticillium* premature ripening and *Phoma* stem canker respectively. *Verticillium longisporum* is a soil-borne vascular pathogen, which infects the plant roots by direct penetration or through open wounds. The pathogen enters the vascular system and then moves upwards in the xylem vessels. Resulting from infection, premature ripening of the seed may occur. *Phoma lingam* (*Leptosphaeria maculans*)-infections are initiated in autumn, when leaves become infected by airborne ascospores. The fungus grows from the lesions down the petiole to the stem base, where it causes canker and subsequent yield loss. The cabbage root fly, which appears in three generations in spring, summer and fall, is thought to be a relatively new pest of oilseed rape. Female flies generally lay their eggs in the soil alongside host plants. Emerging larvae feed on main and lateral roots, which can lead to severe physical damage. Apart from directly harming the plant, root feeding may provide openings for fungal infection. Furthermore plant defence towards fungal pathogens possibly will be impaired, thus accelerating infection and disease progress.

Between 2005 and 2008, several field and greenhouse experiments were performed to determine the impact of root damage, caused by the cabbage root fly, on infections and symptom development of oilseed rape by *Verticillium longisporum* and *Phoma lingam*. In the field, mesh was used to prevent certain plots from root fly attack in autumn and/or spring. In greenhouse trials, root fly infestation was adjusted by laying eggs on to the root collar. The results obtained showed that root damage in autumn facilitate infections with *Verticillium longisporum.* The effect was most pronounced in moderately susceptible cultivars (towards *Verticillium*) and when soil inoculum was low. In contrast, cabbage root fly damage had little effect on *Verticillium* wilt in highly susceptible cultivars.

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Biocontrol of *Sclerotinia sclerotiorum* and *Verticillium longisporum* by the mycoparasite *Microsphaeropsis ochracea*

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Abstract: *Microsphaeropsis ochracea* is a newly discovered mycoparasitic species of Coelomycetes that was first isolated in the 1990s from dead apple leaves originating from Canadian apple orchards (Bernier *et al.*, 1996). By using *M. ochracea in vitro* and field experiments, it is possible to control *Botrytis squamosa*, *Rhizoctonia solani* and *Venturia inaequalis* (Carisse *et al.*, 2000; 2001; 2006).

The antagonistic effect of *M. ochracea* against the oilseed rape pathogens *Sclerotinia sclerotiorum* and *Verticillium longisporum* was investigated in laboratory and field experiments. In laboratory investigations we showed that the germination rates of sclerotia and microsclerotia were reduced by *M. ochracea* treatments. Decrease of germination strongly depended on *M. ochracea* inoculum density, the incubation temperature and the duration of incubation. In order to check the effect of *M. ochracea* on the two oilseed rape pathogens under field conditions we designed two long-term field experiments under an oilseed rape monoculture and an oilseed rape – winter wheat rotation. *M. ochracea* was applied as a formulated product (2,5 x 10^9 spores/g) in autumn before sowing and in spring before the start of stem extension at application rates of 1 kg/ha and 2 kg/ha.

The results indicated lower infestations of rape plants with *S. sclerotiorum* in *M. ochracea* treated plots than in untreated plots.

Key words: *Microsphaeropsis ochracea*, biological control, *Sclerotinia sclerotiorum*, *Verticillium longisporum*

Introduction

In the last 20 years rape growing in the European Union has increased dramatically. Consequently, crop rotations have become more narrow and in some regions rape is grown in some fields every second year. Because of this and the long flowering periods associated with oilseed rape, it is difficult to find the optimal time to apply fungicide treatments to control *S. sclerotiorum*. In such cases, biological fungicides like Contans WG (based on the mycoparasite *Coniothyrium minitans*) can be useful to decontaminate the soil and reduce inoculum density.

Because of intensive rape cultivation, *V. longisporum* has also become an important pathogen of oilseed rape. *V. longisporum* produces microsclerotia on plant residues that are incorporated in the soil by soil cultivation (Zeise & Steinbach, 2004). The microsclerotia are the primary inoculum source and can survive in the soil for several years (Heale & Karapapa, 1999). Because common fungicides are not effective or not registered for use against *V. longisporum* it is difficult to control the pathogen under field conditions. Therefore, the development of biological fungicides similar to Contans WG for the decontamination of the soil from *V. longisporum* microsclerotia would be useful to protect plants from infection with this pathogen.

Material and methods

Laboratory experiments

Growing of Coniothyrium minitans *and* Microsphaeropsis ochracea

C. minitans and *M. ochracea* were grown on oat meal agar at 20 °C, under 16 h of light and 8 h of darkness for three weeks. Spores were harvested by destroying the pycnidia with sterile glass slides and were collected by washing the petri dishes with sterile water.

Sclerotinia sclerotiorum

Sclerotia of *S. sclerotiorum* strain 1.5 (Pathogen collection, Department of Crop Sciences, University of Göttingen) were produced on PDA at 20 °C, under 16 h of light and 8 h of darkness. After three weeks sclerotia were collected from PDA, dried and stored in a fridge. They were inoculated with *M. ochracea*, *C. minitans* and a mixture of both mycoparasites, by dipping them in adjusted spore solutions with $1x10^6$ spores/ml under sterile conditions. For a constant humidity during incubation, 20 sclerotia per replication were put on filter paper placed on water agar. Incubation was done in darkness at different temperatures. The parasitism of sclerotia was checked by viability assays. Sclerotia were transferred to sucrose-salt media (Hedke, 1992) and incubated for 11 d at 20 °C. After this time, sclerotia with yellow halos were assessed as germinated and sclerotia without yellow halos were assessed as parasitized/dead sclerotia.

Verticillium longisporum

Microsclerotia of *V. longisporum* strain V.l 43 (Pathogen collection, Department of Crop Sciences, University of Göttingen) were grown in a rye flour sand mixture. Sterile rye flour sand mixture was inoculated with a V.l 43 spore and mycelia solution, mixed with a sterile scoop and incubated for 21 days at 20 °C in darkness. The microsclerotia-sand mixture was dried on a sterile bench and microsclerotia were separated from the sand by sieving with 315 and 200 µm mesh sized sieves. To check possible parasitism of microsclerotia by *M. ochracea*, microsclerotia with sizes of 200-315 µm were put on filter paper discs placed on water agar and inoculated with 5 µl *M. ochracea* spore suspension with spore densities of $1x10^4$ and $1x10^6$ spores/ml. After incubation for 6, 8 and 10 d, microsclerotia were transferred to vegetable agar (100ml vegetable juice, 15 g Agar, 2g $CaCo_3$, 800 ml H_2O). After incubation for 7 d microsclerotia were checked for viability. Micosclerotia that germinated produced another generation of microsclerotia on vegetable agar. Microsclerotia that did not germinate produced only white mycelia that was identified as *M. ochracea*.

Field trials

To check the biological control of *S. sclerotiorum* and *V. longisporum* by *M. ochracea*, two long term field trials were designed with the following crop rotation sequences winter rape - winter rape and winter wheat – winter rape at two different locations. The application of *M. ochracea* was done using normal spraying technics by desolving the formulated product *Microsphaeropsis* WG ($2.5x10^9$ spores/g, Prophyta GmbH, Malchow, Germany) in water and spraying at a volume of 300 l/ha. Each field trial was designed in a randomized plot design with plot sizes of 15x12 m. The treatment was done with 1 kg/ha and 2 kg/ha *Microsphaeropsis* WG in the autumn and 1 kg/ha and 2 kg/ha Microsphaeropsis WG in autumn and spring. After applying *M. ochracea* to stubble in autumn, the biocontrol agent was incorporated by soil cultivation before the crop was sown as normal. The spring treatment was applied at the beginning of stem extension. The ability of *M. ochracea* in reducing the pathogen inoculum was investigated by checking the disease incidence of the crop at growth

stage 85 for infestations with *S. sclerotiorum* and the disease severity after harvest on rape stubble for the infestation with *V. longisporum*.

Results and discussion

Laboratory experiments

Sclerotinia sclerotiorum

The microscopic studies using GFP labeled *M. ochracea* and DsRed labeled *C. minitans* strains (Bitsadze, 2007 unpublished data) demonstrated the mycoparasitism of *M. ochracea* and *C. minitans* on sclerotia of *S. sclerotiorum*. *M. ochracea* primarily parasitised the rind, while *C. minitans* parasitised the rind and the medulla of sclerotia. In the following we designed *in vitro* experiments for checking possible interactions between *M. ochracea* and *C. minitans* in parasitising sclerotia from *S. sclerotiorum*.

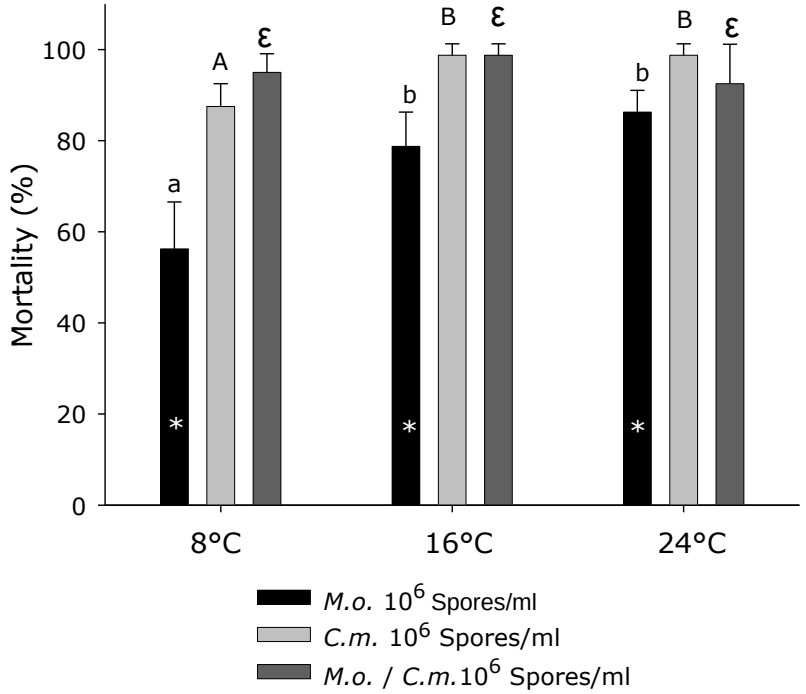

Figure 1. Mortality of sclerotia from S. sclerotiorum after inoculation with M. ochracea (M. o.), *C. minitans* (C. m.) and a mixture of *M. ochracea* and *C. minitans* (M. o./C. m.) depending on incubation temperature 8, 16, 24 °C; Visual check 18 dpi; asterix: statistical differences between the treatments within the temperature; different letters: statistical differences between the temperature within one treatment; n = 4 $p \leq 0.05$.

Within all temperature treatments we detected significant lower mortality rates of sclerotia to single *M. ochracea* treatments in comparison to *C. minitans* and the mixture (*C. minitans* and *M. ochracea*) treatments (Figure 1). This indicated that *C. minitans* was a stronger mycoparasite on sclerotia of *S. sclerotiorum* in comparison to *M. ochracea*. By comparing the separate treatments between the different temperatures we detected increasing

mortality rates within the *M. ochracea* and the *C. minitans* treatment with increasing temperatures, but we did not detect similar effects within the mixture treatment (Figure 1). By mixing *C. minitans* with *M. ochracea,* sclerotia could be degredated a wide range of temperatures because spores of *C. minitans*, the stronger mycoparasite on sclerotia, have lower germination rates at temperatures below 10 °C, and mycelia of *M. ochracea* starts growing at 5 °C indicating that sclerotia of *S. sclerotiorum* germination was inhibited at lower temperatures (Trutmann *et. al.*, 1980; Carisse & Bernier, 2002).

Verticillium longisporum

In preliminary studies with sterile rape stems we detected an inhibition of mycelial growth and microsclerotia formation by inoculating rape stems with *M. ochracea*. In subsequent experiments we designed *in vitro* experiments with single microsclerotia fractions for detecting the mycoparasitism of *M. ochracea* on microsclerotia of *V. longisporum* depending on incubation time and incubation temperature.

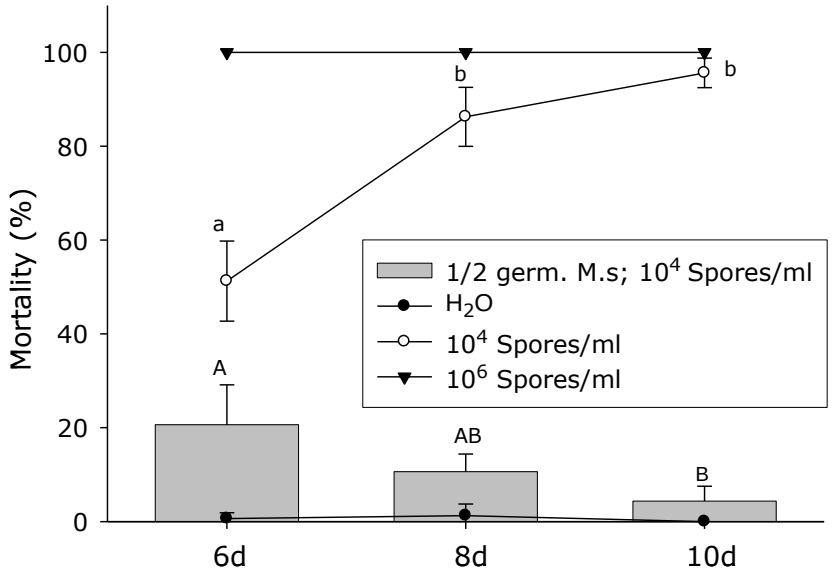

Figure 2. Mortality of *V. longisporum* microsclerotia depending on incubation time (6, 8, 10 days) and *M. ochracea* spore density (1 x 10^4 and 1 x 10^6 spores/ml); Grey bars: partly germinated microsclerotia (%) after treatment with 1 x 10^4 *M. ochracea* spores/ml; Dots and triangle: Dead microsclerotia after treatment with water 1 x 10^4 and 1 x 10^6 spores/ml; different letters: statistical differences between the incubation times; incubation temperature 20 °C; n = 4, $p \leq 0.05$

By treating *V. longisporum* microsclerotia with a spore density of 1 x 10^6 *M. ochracea* spores/ml, microsclerotia were completely degredated after 6 days of incubation. By inoculating microsclerotia with 1x 10^4 *M. ochracea* spores/ml we detected increasing mortality rates with increasing incubation times, and also a decreasing amount of partly germinated microsclerotia (Figure 2). This indicates that the mycoparasitism of *V. longisporum* microsclerotia depended on *M. ochracea* spore density and the incubation time. Furthermore, incubation temperature played an important role in the mycoparasitim of

V. longisporum microsclerotia by *M. ochracea*. After 6 d of incubation microsclerotia were completely inhibited from germination at incubation temperatures over 16 °C. At incubation temperatures below 16 °C the mortality rates decreased and the number of partly germinated microsclerotia increased (data not shown). Similar effects were described by Carisse *et al.*, 2001 where studies indicated that the incubation time and the incubation temperature played an important role in high mycoparasitic efficacy of *M. ochracea* on fungal resting structures.

Field experiments

In early summer at growth stage 85, disease incidence of oilseed rape plants with *S. sclerotiorum* were assessed in the rape-rape field trial at two locations (Göttingen and Dummerstorf). In Göttingen we observed a higher disease incidence of up to 20%, compared to Dummerstorf with a disease incidence of 13%. In Göttingen we found a lower sclerotinia disease incidence in the *M. ochracea* treated plots in comparison to the untreated control, but no differences were found between 1 and 2 kg/ha *M. ochracea* treatment plots and the different temporal treatments.

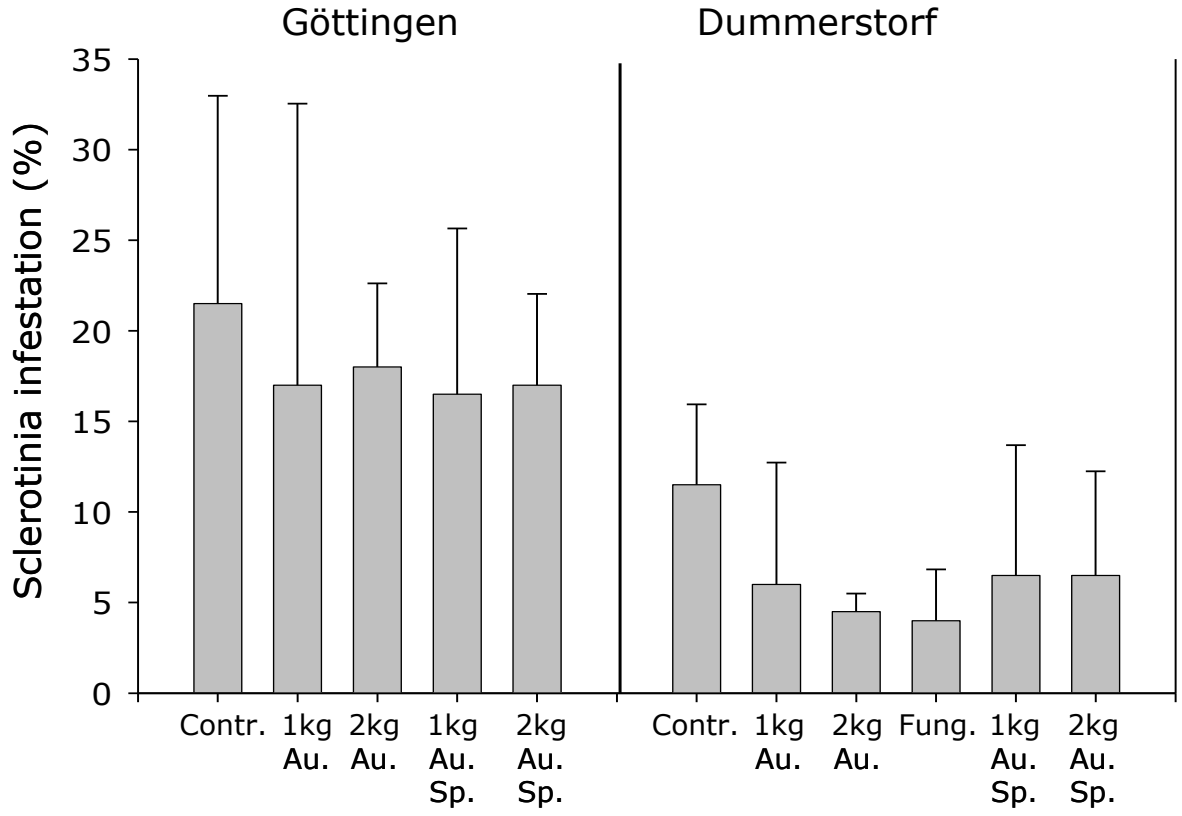

Figure 3. *Sclerotinia* disease incidence (%) at growth stage 85 in Göttingen and Dummerstorf; Contr.: untreated control; 1 kg, 2 kg Au.: 1 and 2 kg/ha treatment in autumn; Fung.: Fungicide treatment at growth stage 65; 1 kg, 2 kg Au., Sp.,: 1 and 2 kg/ha treatment in autumn and spring

In contrast to Göttingen an additional fungicide treated variant was designed in Dummerstorf. The lowest disease incidence was observed in the fungicide treatment and the 2 kg/ha *M. ochracea* treatment in autumn compared to the control. In the other treatments

(1 kg/ha *M. ochracea* in autumn; 1 and 2 kg/ha *M. ochracea* in autumn and spring) we observed a higher *S. sclerotiorum* disease incidence compared to the fungicide and the 2 kg/ha *M. ochracea* treatment in autumn.

However, no differences were found between treatments with respect rape stubble infected with *V. longisporum* after harvest at the described field experiments.

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The influence of *Trichoderma* species on *Leptosphaeria maculans* and *L. biglobosa* growth on agar media and in oilseed rape plants

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Abstract: The ascomycetous fungi *Leptosphaeria maculans* and *L. biglobosa* cause stem canker of crucifers – one of the most damaging diseases of oilseed rape in Poland and worldwide. The pathogens are responsible for major yield losses, which can be economically significant especially in the areas of intensive rapeseed cultivation in Europe, Australia and Canada. The pathogens are observed every year, with a higher proportion of *L. maculans* in West Europe and predominance of *L. biglobosa* in Central and East Europe. The disease is controlled with chemical sprays applied mostly in autumn. At many locations and years one autumn spray is insufficient to fully control the disease. Recently, great efforts are undertaken to study the possibility of biological control using antagonistic and hyperparasitic fungal species. It is postulated to use their potential to combat plant pathogens and use these organisms in integrated pest management technologies to reduce the amount of pesticides introduced to environment.

The aim of this work was to examine possibilities of biological control of *L. maculans* and *L. biglobosa* using hyperparasites from the genus *Trichoderma* obtained from oilseed rape and yellow lupine plants as well as from soil. Tested isolates belonged to *T. atroviride, T. hamatum, T. harzianum, T. koningii* and *T. longibrachiatum.* The experiments were performed under laboratory conditions using dual cultures as well as under glasshouse conditions using oilseed rape plants.

Trichoderma isolates caused significant decrease of growth rate, especially of *L. biglobosa.* On the other hand *L. maculans* showed antibiosis activity and caused decreased growth rate of *T. longibrachiatum*. The species of *Trichoderma* differed in their hyperparasitic effects towards *Leptosphaeria* spp. The highest decrease of *Leptosphaeria* spp. growth rate was caused by *T. atroviride*. Covering of cotyledons with *Trichoderma* spores was proved to serve as protective treatment against *Leptosphaeria* species. The influence of isolates from oilseed rape was significantly greater than the one originating from lupine.

Key words: *Trichoderma* sp., *Leptosphaeria* sp., oilseed rape, biological control

Introduction

The ascomycetous fungi *Leptosphaeria maculans* (Desm.) Ces. et de Not. and *Leptosphaeria biglobosa* Nov. are damaging pathogens of oilseed rape (*Brassica napus* ssp. *oleifera*) and other Brassicaceae plants worldwide. *L. maculans* causes *Phoma* leaf spotting and stem canker (blackleg) and is responsible for major yield losses, which can be economically significant especially in the areas of intensive rapeseed cultivation in Europe, Australia and Canada (West *et al.*, 2001 and 2002; Fitt *et al.*, 2006). In Poland – in contrast to West Europe – the predominant species is *L. biglobosa,* considered as less aggressive (Jedryczka and Lewartowska, 2006).

Both pathogens survive on plant debris. Pseudothecia – fungal fruiting bodies of the perfect stage – are formed on infected stubbles from the previous season. Epidemics are initiated in the autumn by airborne ascospores released from infected debris. These ascospores infect leaves, produce *Phoma* leaf spots and then the pathogen grows down the leaf petiole into the stem (Hammond *et al.*, 1985; West *et al.*, 1999 and 2001). Vast colonization of the

stem base and root collar may lead to severe plant damage or even plant death. The disease is enhanced by high humidity and mild temperatures. Optimized fungicide spray timing helps in controlling stem canker and protecting the crops from *Leptosphaeria* spp. (Gladders *et al.*, 1998; West *et al.*, 2000; Huang *et al.*, 2005).

Chemical treatments however are not the only way to combat pathogens. Another approach is to apply antagonists as biological control agents. Until now there were only few publications about the influence of antagonistic fungi on *Leptosphaeria* spp. pathogenic to oilseed rape. Some experiments revealed that treatment of oilseed rape seeds with *Bacillus amyloliquefaciens* resulted in significant protection against *L. maculans* (Danielsson *et al.*, 2007). Also the use of *Paenibacillus polymyxa* was *in vitro* highly inhibitory to the growth of *L. maculans* (Kharbanda *et al.*, 1999).

Members of the genus *Trichoderma* are among the most potent biocontrol agents applied against plant pathogens (Elad *et al.*, 1994). These organisms are saprophytes and hyperparasites towards other fungi. They are prolific producers of extracellular proteins and are known for their ability to produce enzymes that degrade cellulose and chitin. The mycolytic activity of these enzymes is considered as a major factor of the hyperparasitic mechanism. Species from the genus *Trichoderma* are able to increase resistance of plants to biotic and abiotic stresses, because they are activating Induced Systemic Resistance (ISR) (Chernin *et al.*, 2002). Root colonization by *Trichoderma* species also frequently enhances root growth and development, crop productivity and the uptake and use of nutrients (Chet *et al.*, 1997). Using these organisms to cope with phytopathogens like *Leptosphaeria* spp. can be more effective and safer for the environment than chemical treatments.

The aim of this work was to analyse possible antagonistic effects of five *Trichoderma* species against the phytopathogenic fungi *L. maculans* and *L. biglobosa*. Examined fungal isolates originated mainly from oilseed rape. Experiments were performed both *in vitro* (dual cultures) and *ad planta*.

Material and methods

Fungal isolates used in this study

The isolates of phytopathogenic and antagonistic fungi originated from the culture collection of the Institute of Plant Genetics, Polish Academy of Sciences, Poznan, Poland (Table 1). The testing was done using five isolates of the oilseed rape pathogens *L. maculans* and *L. biglobosa,* respectively. The pathogens were collected at three different sites (Cerekwica, Pawlowice, Zielecin), located in the region of Great Poland (central-west part of Poland) during three seasons (2004-2006). Testing of potential biocontrol activity was performed using 10 isolates belonging to five *Trichoderma* species. Six fungal strains, including all isolates of *T. harzianum, T. koningii* and *T. longibrachiatum* were obtained from oilseed rape plants (*Brassica napus*). All isolates of *T. atroviride* originated from soil taken from a glasshouse experiment with oilseed rape plantlets grown in peat compost. The isolate of *T. hamatum* was obtained from yellow lupine (*Lupinus luteus*).

Table 1. The origin of fungal isolates used in this study

Pathogens				**Antagonists**			
Leptosphaeria species	Symbol	Origin	Year of isolation	*Trichoderma* species	Symbol	Origin	Year of isolation
L. maculans	LMC101	Cerekwica Poland	2004	*T. koningii*	TE2	Lublin Poland	2006
	LMC102			*T. hamatum*	TH7	Ukraine	2005
	LMC103			*T. harzianum*	T13	Zielecin Poland	2006
	LMC104	Pawłowice Poland	2005		T4B		
	LMC105		2006		T3B		
L. biglobosa	LBC101	Cerekwica Poland	2004		T1S		
	LBC102	Pawłowice Poland	2004	*T. longibrachiatum*	TLB1	Poznań Poland	2008
	LBC103			*T. atroviride*	TA1		
	LBC104		2005		TA2		
	LBC105		2006		TA3		

Growth rate of* Leptosphaeria *spp.
Growth rate of *Leptosphaeria maculans* and *L. biglobosa* isolates was examined on 9 cm Petri dishes on PDA medium at 25 °C in darkness, using 3 replicates.

Dual culture experiment
Each pathogenic isolate was inoculated with a 5 mm diameter disc. Subculturing was done using 9 cm Petri dishes with 20 ml of PDA medium. Isolates were placed on a medium, 1 cm from the edge of a plate. Each Petri dish was co-inoculated on the opposite side with the studied *Trichoderma* isolates. The growth inhibition of the tested fungi was evaluated using a protocol described by Manka (1974). In this scale the results can range from –8 to +8, where 0 means no growth inhibition and +8 indicates total growth inhibition of a pathogenic fungus by an antagonist. Three repetitions of each test were made and evaluations were done every day for 2 weeks.

Cotyledon test
Spores were produced on V8-agar medium at 25 °C. Spore concentration was adjusted to $1x\ 10^7$ spores/ml. Ten to fourteen day old plants were inoculated using 5 plants per replicate. Each half of a cotyledon was punctured with a needle. Antagonistic isolates were inoculated using 10 µl droplets of spore suspension. After inoculation, trays were covered with plastic lids and kept in darkness for 48 hours. Afterwards, the inoculated plants were transferred into a growth chamber and kept at 20 °C. Co-inoculation with phytopathogenic isolates was made: 1) on the same day and 2) four days after inoculation with an antagonist. Every tested variant was studied using three replicates. Plant material consisted of three high yielding, double low and open pollinating varieties of winter oilseed rape. The varieties had different origin: Bosman was obtained by Plant Breeding Strzelce, Poland, cv. Brise – by Deutsche Saatveredelung, Germany and cv. Californium originated from Monsanto, France. These cultivars were officially registered in Poland by the Central Station for Variety Testing

(COBORU, Slupia Wielka). Variety Californium is regarded as more resistant to *Phoma* leaf spotting and stem canker caused by *L. maculans*, than the other rapeseed cultivars used.

Fourteen days after plant inoculation the disease symptoms were scored using the IMASCORE rating scale:
0 – no necrotic spots;
1 – small necrotic spots up to 1 mm;
2 – necrotic spots up to 3 mm;
3 – necrotic yellow-brown spots bigger than 3 mm;
4 – grey-green tissue collapse up to 5 mm, without pycnidia;
5 – grey-green tissue collapse up to 5 mm with pycnidia;
6 – grey-green tissue collapse bigger than 5 mm, with more than 10 pycnidia.

Results and discussion

***Growth rate of* Leptosphaeria spp.**

Leptosphaeria biglobosa isolates formed regular, circular colonies. From the very start the growth rate of this pathogen was faster than for *L. maculans*. The colonies of *L. maculans* were often irregular. During the first three days the mean growth rate of *L. biglobosa* on PDA medium was 0.7 mm per day, whereas the isolates of *L. maculans* were growing five times slower. During the following ten days the growth rate was higher; the mean for *L. biglobosa* was 3.02 mm per day and for *L. maculans* it was 2.2 times slower (1.37 mm per day). The colonies reached the edge of a 9 cm Petri dish after 14 days of growth at 25 °C. The average growth rate of *L. biglobosa* isolates was 2.85 mm per day. Growth rate of *L. maculans* was more than twice as low and on average, it reached 1.07 mm per day (Table 2).

Table 2. Growth rate (mm/day) of *Leptosphaeria* spp. isolates on PDA medium at 25 ºC in darkness

Leptosphaeria species	Day interval						Mean growth rate
	1-3	4-6	7-9	10-11	12-13	14-19	
L. maculans	0.14	1.30	1.37	1.55	1.38	0.52	1.07
L. biglobosa	0.70	2.84	2.47	3.19	3.75	3.70	2.85

Dual culture bioassay experiment

Competitive abilities of *Trichoderma* spp. against pathogenic *Leptosphaeria* isolates were examined in dual culture bioassays on agar. A week after inoculation growth inhibition and mycoparasitism of *Trichoderma* isolates were observed – the plate was green and overgrown with *Trichoderma* with significant production of abundant conidia in pustules over mycelium of *Leptosphaeria* isolates. The growth of the pathogen was strongly inhibited by isolates of all *Trichoderma* species studied.

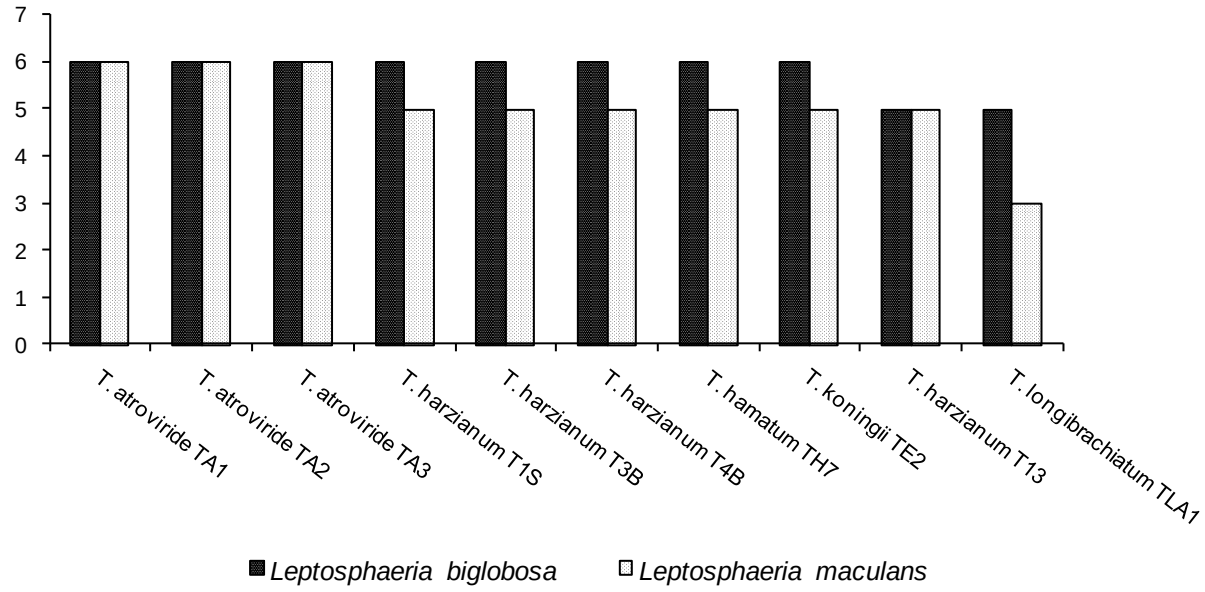

Figure 1. Growth inhibition of *Leptosphaeria maculans* and *L. biglobosa* in dual cultures with *Trichoderma* spp. (scale according to Manka, 1974, 0 – no, 8 – total growth inhibition)

Three types of interaction between *Trichoderma* species and plant pathogens can be recognized: antibiosis, competition for nutrients and hyperparasitism (Woo *et al.*, 2006). The antibiosis effect was observed in case of *L. maculans*. The two of candidate antagonistic fungi *T. longibrachiatum* and *T. harzianum* caused 2 mm inhibition zones separating them from the pathogenic fungi, what retarded the growth of *L. maculans*. On the other hand growth speed of *T. longibrachiatum* was decreased in bioassays with *L. maculans*. This effect could be caused by secondary metabolites produced by this phytopathogenic fungus (Pedras and Yu, 2008). Fast growing *L. biglobosa* was suppressed relatively more than slow growing *L. maculans*, what could also coincide with higher toxicity of the latter species.

Trichoderma isolates are able to produce antibiotics – inhibitors of other fungi (like 6PAP), several enzymes, which hydrolyse fungal structures and macromolecules such as chitin, cellulose, hemicellulose, beta glucan, xylem and proteins (Lacicowa and Pieta, 1985; Kubicek and Harman, 1998; Cooney *et al.*, 2001). This ability allows *Trichoderma* isolates to utilise the mycelium of *Leptosphaeria* spp. as a source of nutrients. Our biotest experiments confirmed the mycoparasitism of *Trichoderma* species over two *Leptosphaeria* species. All *Trichoderma* species showed the effect of mycoparasitism over *L. biglobosa*. In the case of *L. maculans* the strongest hyperparasitic interaction was observed for *T. atroviride*, *T. hamatum*, and *T. koningii*.

Cotyledon test

Protective abilities of antagonistic fungi against pathogenic *Leptosphaeria* isolates were examined in a cotyledon tests. In the first experiment young oilseed rape plants were inoculated simultaneously with the antagonistic fungi and with phytopathogens. In case of variety Bosman no change was observed between the seedling inoculation solely with the pathogens and the combined inoculation with *Trichoderma* species (Table 3). However, the co-inoculation of *L. maculans* with *T. harzianum* and with *T. atroviride* resulted in decreased disease severity of cv. Brise. Similar effect was observed on cv. Californium co-inoculated

with *L. maculans* and *T. longibrachiatum*. The effect of decreased symptom severity was also found for combined inoculations of *L. biglobosa* with *T. atroviride* and with *T. hamatum.* Plant inoculation using the conidial spores of *L. maculans* without the isolates of *Trichoderma* resulted in strong disease symptoms in all cultivars tested. Similar effect was obtained using the conidiospores of *L. biglobosa.* In this case the spots were necrotic, yellowish-brown and very big. They usually covered whole cotyledons, but no sporulation was observed during the experiment.

Table 3. Disease severity (IMASCORE scale 0-6, Volke 1999) obtained 14 days after co-inoculation of *Leptosphaeria* spp. with different isolates of *Trichoderma* spp.

Leptosphaeria species	*Trichoderma* species used for co-inoculation	Variant of co-inoculation					
		Simultaneous co-inoculation using *Trichoderma* sp. and *Leptosphaeria* sp.			Inoculation with *Trichoderma* sp. 4 days before *Leptosphaeria* sp.		
		Bosman	Brise	Californium	Bosman	Brise	Californium
L. maculans	No co-inoculation	6	6	6	5	6	6
	T. longibrachiatum	6	6	**5***	5	**5**	6
	T. harzianum	6	**4**	6	**4**	6	6
	T. atroviride	6	**5**	6	5	**5**	6
	T. hamatum	6	6	6	5	6	6
L. biglobosa	No co-inoculation	3	3	3	3	3	3
	T. longibrachiatum	3	3	3	3	3	**2**
	T. harzianum	3	3	3	3	3	**2**
	T. atroviride	3	3	**2**	**2**	3	**2**
	T. hamatum	3	**2**	3	3	3	3

* bold numbers indicate the decrease of disease severity as compared to inoculation with *Leptosphaeria* sp. alone

The treatments of oilseed rape seedlings using *Trichoderma* isolates followed by inoculation with pathogenic *L. maculans* and *L. biglobosa* demonstrated protective abilities of all mycoparasitic species with the exception of *T. hamatum* (Table 3). In 7 out of 24 co-inoculation variants a decrease of disease symptoms was demonstrated. In most cases it was one score less comparing to inoculation with conidia of the pathogen only. The most frequent inhibition of pathogen activity was observed for *T. atroviride*. The co-use of this species and *Leptosphaeria* spp. decreased disease symptoms observed on cultivar Brise inoculated with *L. maculans* and on cultivars Bosman and Californium inoculated with *L. biglobosa*. No protective effect of *Trichoderma* sp. was found on cv. Californium treated with *L. maculans*, but in the case of *L. biglobosa* activity of *Trichoderma* was observed for *T. longibrachiatum, T. harzianum* and *T. atroviride* (Table 3). Covering of cotyledons with *Trichoderma* spores

was proved to serve as protective treatment against *Leptosphaeria* species. The results were obtained under conditions conducive both for the pathogen and the hyperparasite. Field tests allowing studies on the *Trichoderma – Leptosphaeria* fungus interaction in natural field conditions are in demand. Experiments in different environments and weather conditions will investigate the potential of *Trichoderma* species as a biological control agent in agricultural practice.

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Blackleg, stem canker

Integrated Control in Oilseed Crops
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SIPPOM-WOSR, Simulator for Integrated Pathogen POpulation Management: a tool to help design and evaluate sustainable strategies to control *Phoma* stem canker on winter oilseed rape at the regional scale

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Abstract: *Phoma* stem canker, also known as blackleg, is a major disease of oilseed rape. Among the different means to control the disease – chemical treatments, agronomic practices and plant genetic resistances – the use of resistant cultivars is the most efficient. Winter oilseed rape cultivars have two types of resistance to *Phoma* stem canker, either specific or quantitative. New specific resistances are extremely efficient but may lack durability. Combining genetic, cultural and chemical control methods at the multiple-years and regional scales could help contain *Phoma* stem canker and preserve the efficiency of specific resistances, while ensuring economic profit for farmers and satisfying the environmental and toxicological exigencies of Integrated Crop Management. Given the considered scales and the number of technical operations that have to be taken into account, it is highly difficult to test disease management strategies using traditional field experiments. A model has been developed to evaluate the agronomic, economic and environmental performances of spatially distributed cropping systems: SIPPOM-WOSR, a Simulator for Integrated Pathogen Population Management, for Winter OilSeed Rape. SIPPOM consists of 5 sub-models simulating i) primary inoculum production, ii) ascospore dispersal, iii) crop growth and attainable yield, iv) dynamics of pathogen population genetic structure, and v) infection and relative yield loss. The output variables are disease severity indices and the associated yield losses, actual yields, gross margins, energetic costs of cultural practices and Treatment Frequency Indices. It also calculates the genetic structure of pathogen populations depending on four evolutionary forces or genetic mechanisms: migration, selection, recombination, and the allele effect. A sensitivity analysis has been carried out to study the sensitivity of the sub-models to parameter variations. It showed that SIPPOM can be confidently used to rank contrasted integrated control strategies. The evaluation of each sub-model revealed correct predictive quality. A comparison between simulated and observed data during the loss of efficacy period – 1994 to 2000 – of the *Rlm*1 specific resistance gene in the centre of France was satisfactory. Nevertheless, the results underlined the interest of introducing virulence costs in SIPPOM. Further simulations were carried out on the 2004-2008 period to assess the behaviour of SIPPOM for realistic field spatial distributions and cropping systems. Results showed that the disease index calculation should be adjusted to improve SIPPOM's predictive quality. After improvements, strategies minimising the severity of *Phoma* stem canker and the risk of specific resistance loss of efficacy will be simulated.

Key words: *Leptosphaeria maculans, Brassica napu,* cultural control, Integrated Crop Management, resistance durability, model simulation

Introduction

Phoma stem canker, also known as blackleg, is a major disease of oilseed rape world-wide. The use of resistant cultivars is the most efficient control method against the disease. Winter oilseed rape cultivars have two types of resistance to *Phoma* stem canker, either specific or quantitative. However, specific resistances may lack durability. To enhance the durability of specific resistances, Integrated Avirulence Management (IAM, Aubertot *et al.*, 2006) consists of limiting the selection pressure exerted on pathogen populations and in reducing the size of pathogen populations. On one hand, it is necessary to rationalise cultivar deployment in space and over time. On the other hand, reduction of pathogen population size should be done by combining other control methods, entailing chemical and cultural control methods. Fungicide treatment can limit primary infection. Cultural practices, such as sowing rate or organic nitrogen application before sowing, have an impact on the leaf area receiving ascospores, and thus on the risk and intensity of infection. Shifting the sowing date can prevent the coincidence between ascospore release and the most sensitive stage of oilseed rape to infection. After harvest, soil tillage can reduce the quantity of primary inoculum by burying infected stubble and preventing pseudothecial maturation, and thus ascospores release. Disease control can be improved by reducing spore flow between fields, for which we need to consider spatial distribution of oilseed rape fields within the landscape. Given the large scales and the number of technical operations that have to be taken into account, it is difficult to test disease management strategies using traditional field experiments.

Our aim was to develop a model (SIPPOM-WOSR, a Simulator for Integrated Pathogen Population Management to manage *Phoma* stem canker on winter oilseed rape), to evaluate strategies combining genetic, cultural and chemical control methods at the regional scale and over several years. Tested strategies should aim at containing *Phoma* stem canker and preserving the efficiency of specific resistances, while ensuring profit for farmers and satisfying the environmental and toxicological exigencies of Integrated Crop Management.

Material and methods

Conception of SIPPOM

The model is based on existing submodels that have been adapted for their use in SIPPOM, such as SimCanker to simulate infection and subsequent yield loss (Aubertot *et al.*, 2004), Anthracnose Tracer to simulate ascospores dispersion (Diggle *et al.*, 2002) or Azodyn-Rape to simulate crop growth (Jeuffroy *et al.*, 2003). New sub-models have been created, based on experimental data, such as the genetic sub-model (simulating the effect of specific and quantitative resistance on infection and evolution of the pathogen population, as well as recombination and allele effect) or the attainable yield prediction sub-model.

Sensitivity analysis and evaluation of SIPPOM

A sensitivity analysis was carried out to study the sensitivity of the sub-models to parameters' variations, under contrasted input variable situations (weather*crop management). Variation of output variables was analysed, as well as the stability of the ranking of contrasted situations when parameters varied.

Each sub-model was independently evaluated, either by using data independent from the one used for parameterization, or by cross-validation. Comparisons between observations and simulations were performed in two realistic situations. The *Rlm*1 specific resistance gene loss of efficacy were observed in the central region of France (1994 to 2000; Rouxel *et al.*, 2003)

and it was simulated in SIPPOM, in a small region of 16 km². Usual rotation and agronomic practices (surveyed during this period) were simulated, and evolutions of frequencies of pathotypes were compared. In addition, a comparison between simulated and measured G2 Disease Index and yields at the field scale was achieved over the 2004-2008 period. Cultural practices, disease severity and genetic sampling were collected over this period in the 16 km² area, where cultivars with a new specific resistant gene (*Rlm*7) had been introduced.

Simulations

Preliminary simulations were carried out to show the potential of SIPPOM in terms of possible use, and to enlighten necessary improvements of the model. A first set of simulations were performed with a simplified 3 km * 3 km landscape with 144 fields representing a systematic WOSR-wheat-barley succession. Two crop managements were tested: one called "integrated", favouring the diminution of disease level (ploughing, early sowing, low density), and the other, called "intensive", maximizing the potential yield (high density), but with only a fungicide treatment against *Phoma*. Four WOSR cultivars were used in simulations, characterized by (i) a specific resistance, (ii) a quantitative resistance, (iii) a specific and a quantitative resistance, (iv) *Phoma* susceptibility. Strategies combining crop management and cultivars were tested.

Results and discussion

Conception of SIPPOM

SIPPOM consists of 5 sub-models (Figure 1) simulating i) primary inoculum production on surface stubble after tillage, ii) ascospore dispersal in the landscape (the model is spatially explicit), iii) crop growth and attainable yield, iv) dynamics of pathogen populations genetic structure, and v) infection and relative yield loss. The output variables are disease severity indices and the associated yield losses, actual yields, gross margins, energetic costs of cultural practices and Treatment Frequency Indices. The model also calculates the genetic structure of the pathogen population depending on four evolutionary forces or genetic mechanisms: migration, selection, recombination, and the allele effect.

Sensitivity analysis and evaluation of SIPPOM

Sensitivity analysis showed high variation of output when parameters vary. However, despite this variation, ranking of contrasted input situations was stable. Moreover, ranking was in agreement with what was expected by experts, which means that SIPPOM can be confidently used to rank contrasted integrated control strategies.

The evaluation of each submodel revealed correct predictive quality. Simulations of the pathotypes frequencies's evolution were consistent with observations (Figure 2), even if the predominance of virulent pathotypes was faster in simulations. Results underlined the interest of introducing virulence costs in SIPPOM, as the pathotypes with the *Avrlm*4 gene persisted in observations, which may be due to a higher fitness (Huang *et al.*, 2006).

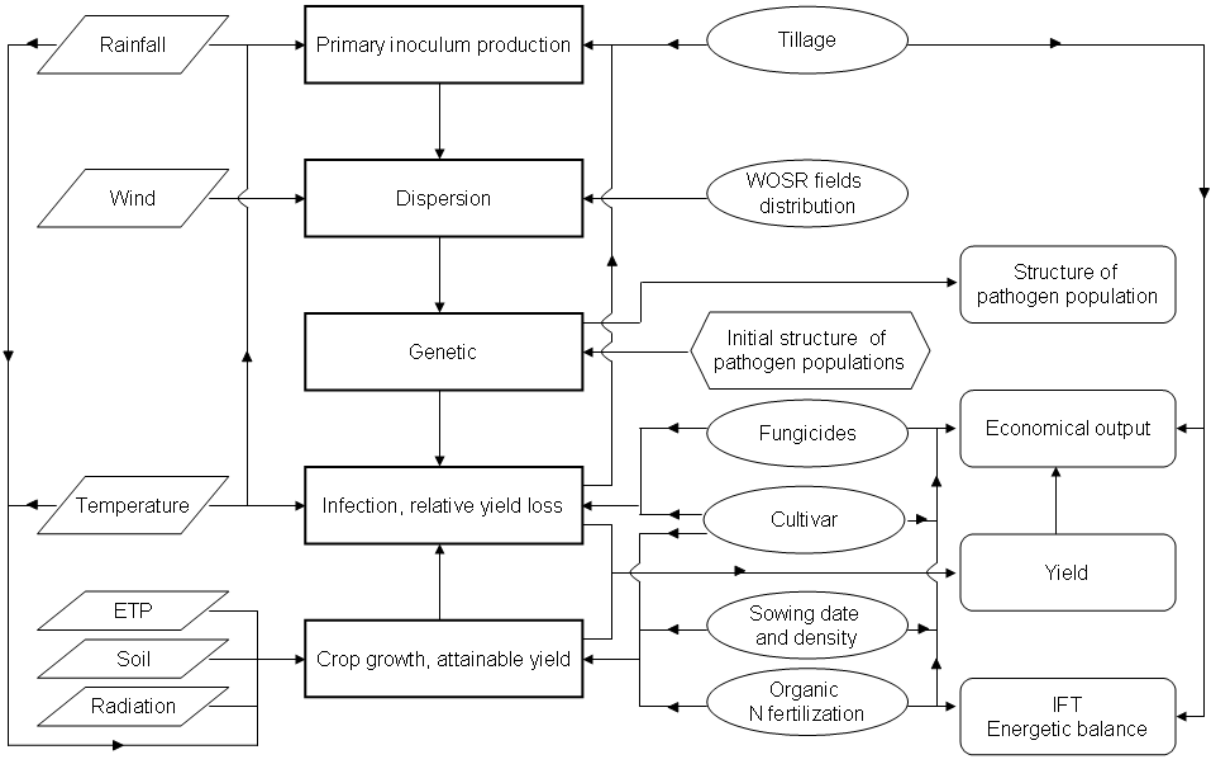

Figure 1. Flow chart of SIPPOM-WOSR. Sub-models are represented in squares, climatic and soil inputs data in diamonds, technical inputs data in ovals, and outputs data in rounded squares. The structure of pathogen populations is an input variable (initial structure), a state variable (simulated each year) as well as an output variable. The quantity of inoculum also depends on the severity of the disease in the previous year (Lô-Pelzer *et al.*, 2008)

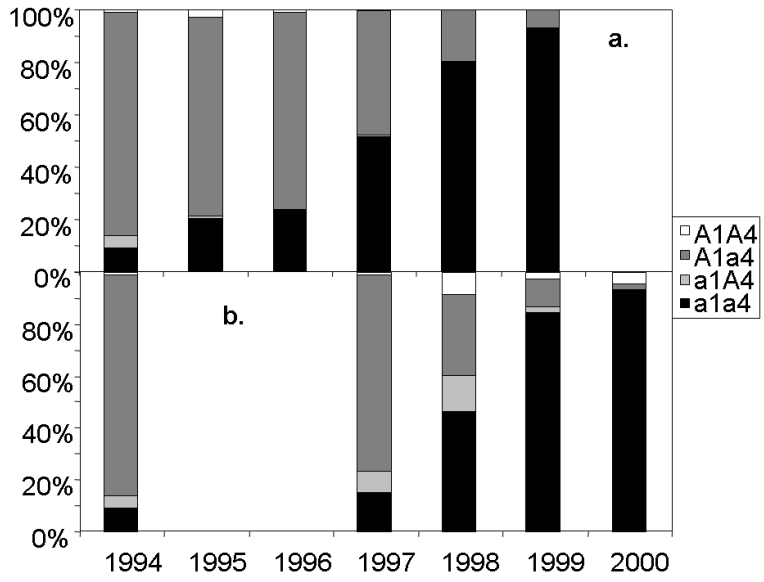

Figure 2. Evolution of the frequencies of the pathotypes associated with the resistance genes *Rlm*1 and *Rlm*4, following the introduction of the specific resistant gene *Rlm*1. Comparison between simulated (a.) and observed (b.) frequencies. A: pathotype with the considered avirulence gene, a: pathotype without the considered avirulence gene.

The comparison between observed and simulated G2 values in the second realistic situation showed a poor prediction (RMSE = 3.1) with an overestimation of the disease index (Bias = 2.4).

Evaluation of the overall model was difficult given the spatial and temporal scales involved, and the number of input and intermediate variables. Presented comparisons were not an evaluation of the predictive quality of the model *sensu stricto*. They assessed the general behaviour of SIPPOM in realistic situations and showed that the evolution of the pathogen population was correctly predicted, but that SIPPOM needed improvement with respect to the calculation of the disease index.

Simulations

Simulations of the simplified landscape indicated that SIPPOM was able to represent the effect of crop management and the effect of the different types of resistant cultivars - as well as their combination and spatial distribution - on disease level and on pathogen population dynamics in terms of virulence. It demonstrated that the application of an integrated crop management extended the durability of the specific resistance efficiency by limiting the pathogen population size to the WOSR fields with a specific resistant cultivar (Figure 3). This could be further explored with SIPPOM.

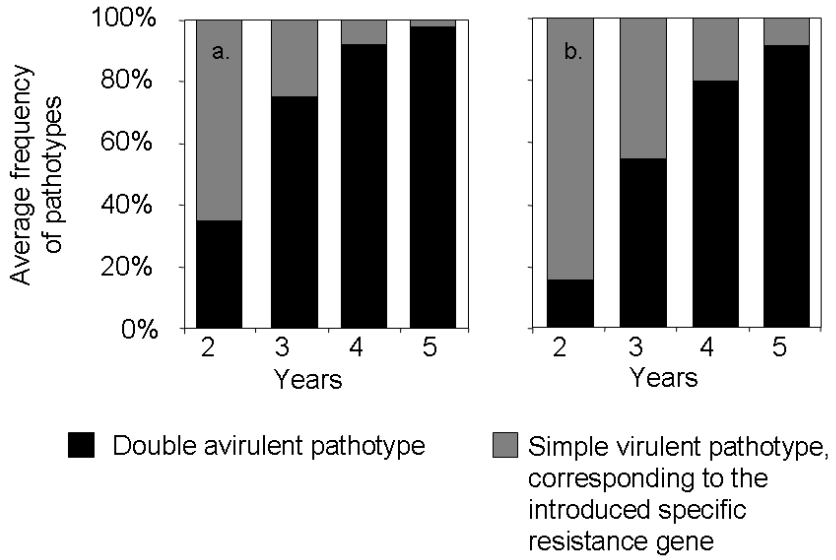

Figure 3. Simulation of the association of an integrated crop management, limiting the pathogen population size (ploughing), to the WOSR fields with the specific resistant cultivar. a. 50%* of WOSR fields with a specific resistant cultivar, 50%* of WOSR fields with a susceptible cultivar, integrated crop management, b. 50%* of WOSR fields with a specific resistant cultivar associated with the integrated crop management, 50%* of WOSR fields with a susceptible cultivar associated with the intensive crop management.

*Proportion of winter oilseed rape fields in a 9 km² landscape with WOSR-Wheat-Barley rotation

Simulations also highlighted necessary improvements of the model, particularly in the simulation of the relationship between the number of ascospores, the formation of leaf-spots and the subsequent canker severity. However, this will need further experiments. Despite these necessary improvements, simulations and sensitivity analysis showed that SIPPOM can

be used to test strategies. Simulations also highlighted new results that cannot be proved without a tool such as SIPPOM. After improvements, simulations in realistic landscape will be performed, to rank strategies reducing disease risk and limiting the risk of loss of efficacy of specific resistances.

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Prediction of *Leptosphaeria maculans – L. biglobosa* pseudothecial maturation in Poland

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Abstract: The fungal pathogens *Leptosphaeria maculans* (Desm.) Ces. et de Not. and *L. biglobosa* Shoemaker & Brun are responsible for *Phoma* stem canker – the disease is regarded as the most damaging to oilseed rape (*Brassica napus* L.) worldwide. In Europe, rapeseed plants are mostly infected in the autumn by ascospores produced in pseudothecia. These fruiting bodies of the perfect stage are formed on dead stems of oilseed rape plants infected in a previous growing season. It was proved that fungicide treatments against these pathogens are more effective when applied during the period of mass ascospore release, which occurs after a rain event following a full pseudothecial maturation. The prediction of the rate of *L. maculans* and *L. biglobosa* fruiting body maturation is therefore an important information for the optimisation of agrotechnical and chemical practices in cultivation of oilseed rape. The prediction of *L. maculans* and *L. biglobosa* pseudothecial maturation in Poland was based on a 9 year dataset (1998-2006), comprising biological observations of fungal development and two basic weather data: mean daily temperature and rainfall, beginning at harvest time of the previous cropping season of oilseed rape. The study concerned 100 site-years with one experiment site per year between 1998 and 2003 and the average of 35.5 sites per year in the later period. From 1998 to 2006 weather data were collected at experiment locations. Since 2006 the average distance from data collection site to a weather station was 13.3 km. The prediction model for pseudothecia maturation hypothesises that the probability of pseudothecial maturation follows a Gaussian distribution, as a function of the number of cumulated days favourable for maturation. The parameterisation of the model led to the following values: minimum daily temperature = 6.0 °C; maximum daily temperature: 29.6 °C; minimum cumulated rainfall over a 12-day period = 4.0 mm and standard deviation of the number of days favourable required for pseudothecial maturation σ_{FD} =1.9 days. The efficiency of the model was greater than 0.77, which suggests that the model can be used in a decision support system.

Key words: oilseed rape, phoma stem canker, mathematical modelling, epidemiology

Introduction

Phoma stem canker, the most damaging disease of oilseed rape, is caused by a complex of two related species of fungal pathogens belonging to the genus *Leptosphaeria*: *L. maculans* and *L. biglobosa* (Shoemaker and Brun, 2001). The former species is regarded as more damaging and is predominant in some countries, e.g. Australia (Salisbury *et al.*, 1995). In contrast, *L. biglobosa* was the predominant pathogen causing stem canker on oilseed rape in Poland (Jedryczka *et al.*, 1994), however, in recent years the population has been gradually changing in favour of *L. maculans* (Karolewski *et al.*, 2002). The main source of infection by these pathogens are ascospores produced in fruiting bodies (pseudothecia) on infected stubbles of oilseed rape from a previous season (Petrie, 1995). Ascospore release is triggered by rainfall; the spores land subsequently on wet leaves and initiate infection. The process of pseudothecial maturation and ascospore release is weather dependent with strong effect of rainfall and temperature (Salam *et al.*, 2003, Toscano-Underwood *et al.*, 2003, Huang *et al.*,

2005). In year 2004, the System for Forecasting Disease Epidemics (SPEC) started operating in Poland. The system is based on monitoring maturation of pseudothecia and ascospore concentration in the air at different locations throughout Poland (Jedryczka *et al.*, 2004). This system was used as a base for the development of a model that predicts the dynamic of pseudothecial maturation and ascospore showers using only two basic weather data: mean daily temperature and summary daily rainfall.

Material and methods

Data collection

The data on pseudothecial maturity were collected from January1998 until November 2006. In the first six years (1998-2003) the data were gathered from one experiment site, in 2004 the number of sites increased to 5 points, and in 2005-2006 data were collected from 44 and 45 points respectively. From each site, once a week, 10 fruiting bodies from 6 pieces of infected oilseed rape stems were examined. In total, 84 thousand samples were examined (Table 1).

For each experiment site, the mean daily temperature and daily rainfall was recorded by the Research Centre for Cultivar Testing - located directly at sampling sites (10 locations) and TRAX Elektronik meteorological stations – located on average in 13.3 km from the sampling sites (34 sampling sites).

Pseudothecial maturation assessment

From 1998 to 2004, the stages of pseudothecial maturation were ascribed to four classes (A-D) and in 2005 and 2006 to five classes (A-E), according to the following characters:

- A – immature pseudothecia with undifferentiated asci;
- B – pseudothecia with partially differentiated asci but without ascospores;
- C – differentiated asci, with less than 8 ascospores or ascospores with less than 4 cells;
- D – mature asci containing 8 ascospores, each with more than 4 cells;
- E – empty asci.

Database preparation

The prediction of *L. maculans – L. biglobosa* pseudothecial maturation in Poland was based on a 9 year dataset (1998-2006), comprising biological observations of fungal development and two basic weather data: mean daily temperature and rainfall, beginning at harvest time of the previous cropping season of oilseed rape. The total number of studied site-years was 100, with one experiment site per year between 1998 and 2003 and the average of 35.5 sites per year in the later period (Table 1). From 1998 to 2006 weather data were collected at the experiment field and since 2006 the data from additional sampling points were associated with weather stations located on average by 13.3 km distance from the experiment site.

Structure of the model

The aim of the model is to predict the dynamic of pseudothecial maturation as a function of climatic variables. The basic principle of the model relies on the concept of accumulation of days favourable for pseudothecial maturation. A day is considered as favourable to pseudothecial maturation if the mean daily temperature is less than the maximum threshold value (θ_{max}) and higher than the minimum threshold value (θ_{min}). The total rainfall for the last n_r days has to be greater or equal to another threshold (r_{min}).

It is hypothesised that the probability of pseudothecial maturation approximately follows a bell-shaped distribution as a function of the number of cumulated favourable days to

maturation. This distribution is represented by a Gaussian function where N_{FD} is the expected number of favourable days required for pseudothecial maturation; and σ_{FD} is the standard deviation of the number of favourable days required for pseudothecial maturation.

A binary variable, denoted DFM(i), describes if the day i is favourable to maturation (DFM(i) = 1) or not (DFM(i) = 0). Another variable, denoted CDFM(d), represents the cumulated number of days favourable to maturation after harvest. The proportion of mature pseudothecia is then calculated using the Gaussian distribution (expected value: N_{FD}, standard deviation: σ_{FD}).

Table 1. Number of biological and meteorological observations used to estimate the parameters of the model

Season	Number of pseudothecial maturation experiment sites	Number of pseudothecial maturation observations	Number of meteorological stations	Number of meteorological observations (temperature/rainfall)
1998	1	840	1	196
1999	1	840	1	196
2000	1	840	1	196
2001	1	840	1	196
2002	1	840	1	196
2003	1	840	1	196
2004	5	4200	5	980
2005	44	36960	44	8624
2006	45	37800	45	8820
Total	100	84000	100	19600

Parameterisation and evaluation of the model

Initial values of four parameters were obtained from published data (Salam *et al.*, 2003). The parameters were as follows: θ_{max} = 22 °C; θ_{min} = 4 °C; r_{min} = 4.0 mm; n_d = 7 days; N_{FD} = 43 days. The initial value of σ_{FD} was defined to ensure that the simulated proportion of mature pseudothecia at harvest was quasi-null. Most of these parameters were estimated in Australian conditions, which are very different from Polish conditions, so a re-parameterisation was performed using Polish data. A first parameterisation was performed with the use of dataset from years 1998-2005 and second with the use of 2006 year data.

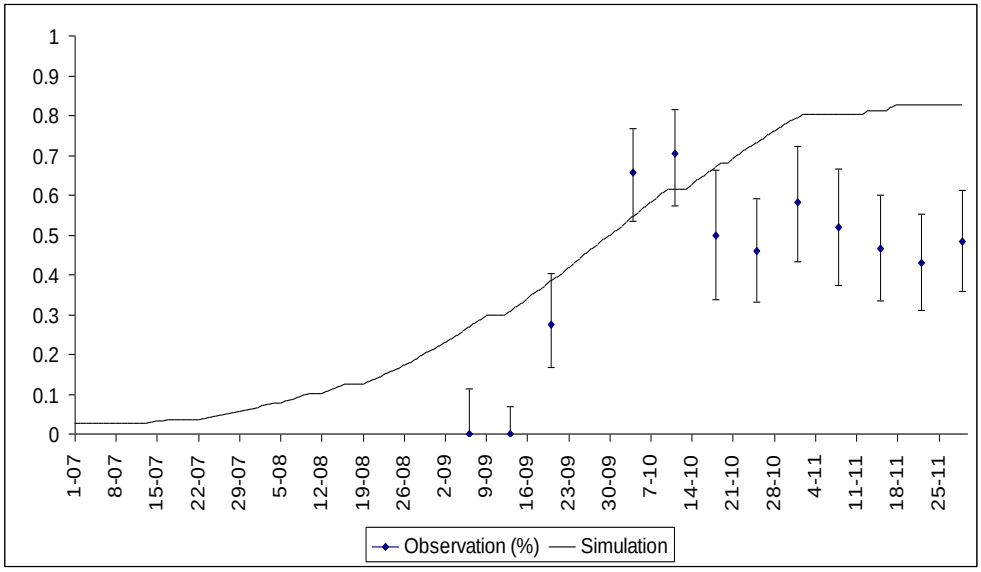
The assessment of the fitting quality of the model was characterised using Bias and the Root Mean Squared Error (RMSE) as statistical criteria. A cross-validation was performed to assess the predictive quality of the model. This quality of prediction was characterised using the efficiency and the Root Mean Squared Error of Prediction (RMSEP) criteria.

Results and discussion

After parameterisation using data from years 1998-2005 the model was systematically ahead of observations (Figure 1). The best prediction quality was obtained by adjusting five parameters: N_{FD}, $\sigma_{FD,}$ $\theta_{max,}$ θ_{min} and n_d. The minimum value of $RMSEP_{CV}$ for the proportion of

mature pseudothecia was 0.41 and the associated Bias was 1.96 10^{-1}. Selected values were: maximum mean daily temperature threshold θ_{max} = 20.3 °C; minimum mean daily temperature threshold θ_{min} = 4.9 °C; minimum total rainfall within the last n_d days r_{min} = 4.0 mm; n_d = 14 days; expected number of days favourable required for pseudothecial maturation N_{FD} = 65 days; standard deviation of the number of days favourable required for pseudothecial maturation σ_{FD} = 34 days (Table 2).

a



b

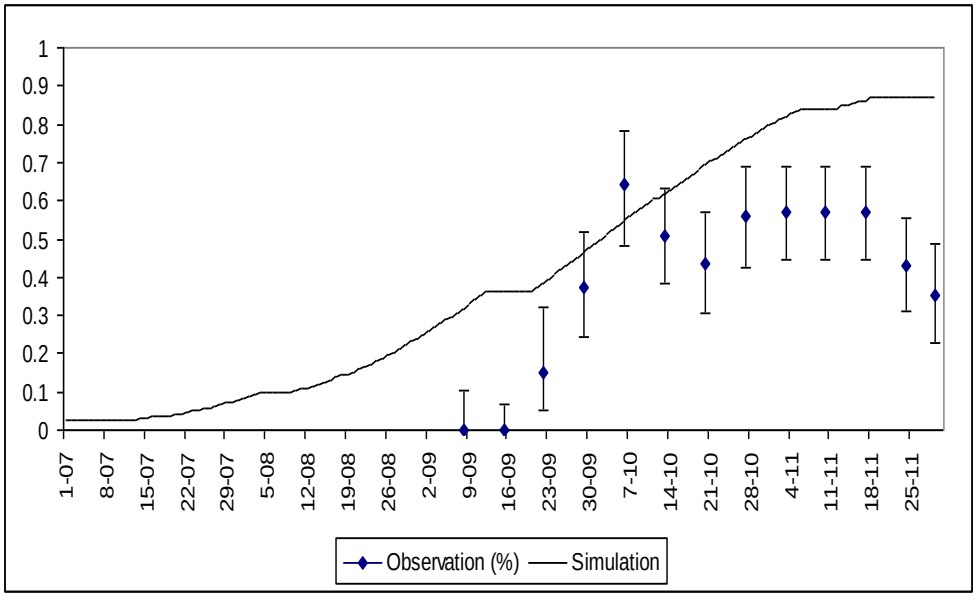


Figure 1. Examples of the predictive quality of the model with parameters adjusted to Polish conditions at two experiment sites in the year 2005: a) Tarnow (south-west Poland), b) Rarwino (north-west Poland). The lines demonstrate the simulated proportion of mature pseudothecia. The dots point out the observations. Error bars are the 95% confidence intervals. Parameters were estimated using 1998-2005 data.

The parameterisation of the model was repeated using the database from years 1998-2006. In this case, simulations were much closer to observations (Figure 2). The best prediction quality was also obtained by adjusting five parameters: N_{FD}, $\sigma_{FD,}$ θ_{max}, θ_{min} and n_d. The minimum value of $RMSEP_{CV}$ for the proportion of mature pseudothecia was 0.28, and the associated Bias was -1.50 10^{-1}. The efficiency of the model was greater than 0.77, and RMSEP was 0.14. The final parameter values were: maximum mean daily temperature threshold θ_{max} = 29.7 °C; minimum mean daily temperature threshold θ_{min} = 6.0 °C; minimum total rainfall within the last n_d days r_{min} = 4.0 mm; n_d = 12 days; expected number of days favourable required for pseudothecial maturation N_{FD} = 65 days; standard deviation of the number of days favourable required for pseudothecial maturation σ_{FD} = 1.9 days (Table 2).

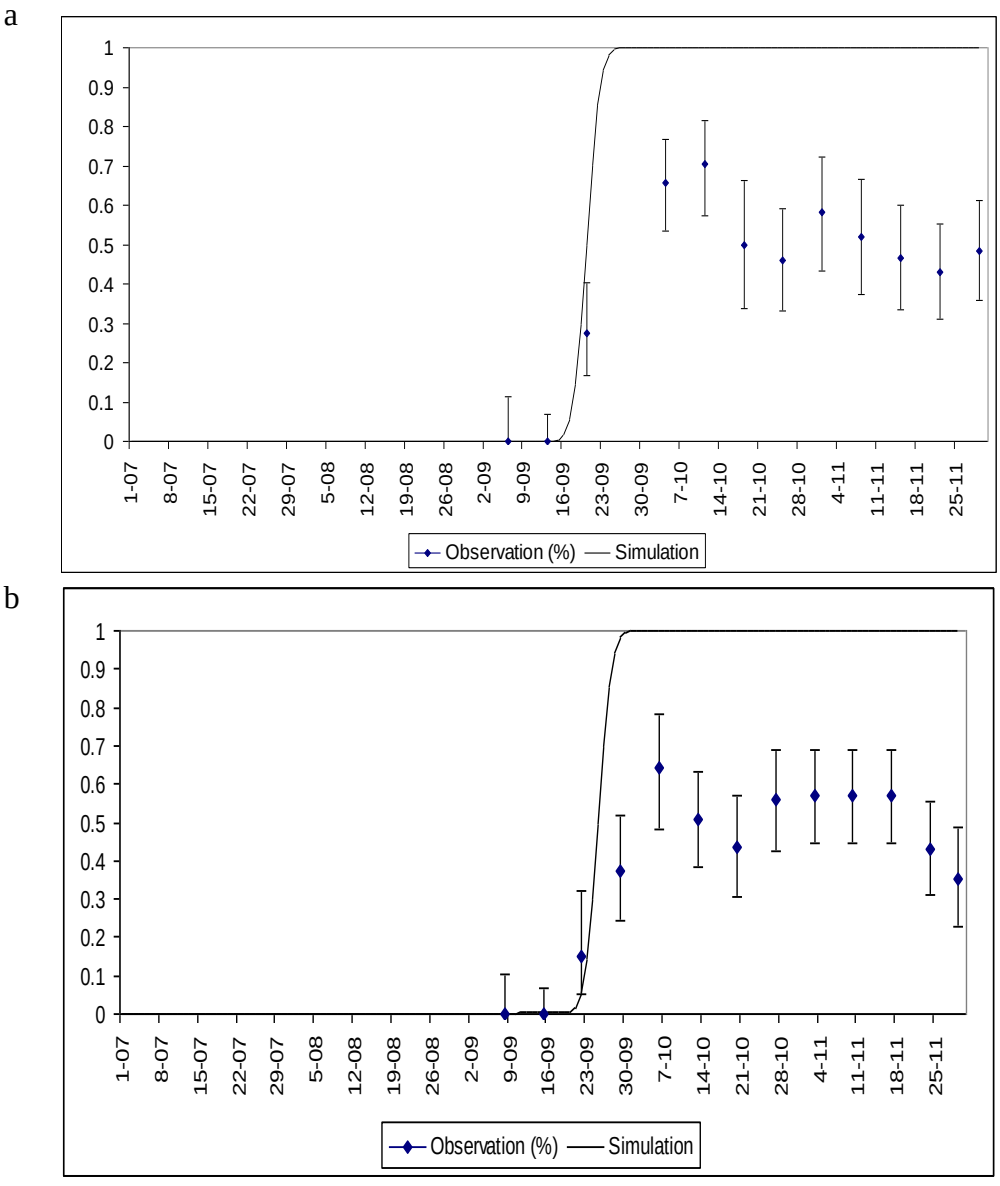


Figure 2. Examples of the predictive quality of the model with parameters adjusted to Polish conditions in two experiment sites in the year 2005: a) Tarnow (south-west Poland), b) Rarwino (north-west Poland). The lines are the simulated proportions of mature pseudothecia. The dots point out observations. Error bars are the 95% confidence intervals. Parameters were estimated using 1998-2006 data.

Table 2. The values obtained after parameterisation with different datasets

Parameter	Initial values from literature	Values obtained after parameterisation with the 1998-2005 dataset	Values obtained after parameterisation with the 1998-2006 dataset
θ_{max} (ºC)	22	20.29	29.66
θ_{min} (ºC)	4	4.88	6
r_{min} (mm)	4	4	4
n_d (d)	7	13.99	12.01
N_{FD} (d)	43	65	65
σ_{FD} (d)	5	33.92	1.89

The dynamic of pseudothecial maturation is a crucial part of *Phoma* stem canker epidemics. The process can be predicted with the help of a mathematical model, such as Blackleg Sporacle – the first and very successful attempt undertaken in Australia (Salam *et al.*, 2007). The biological and meteorological data obtained from numerous sampling points all over Poland and a long range of consecutive years were used to elaborate a mathematical model based on SimMat model (Aubertot *et al.*, 2006). However, the whole model was re-parameterized using Polish data that caused modifications in parameter values. It allowed to improve the predictive quality of the model in the climatic zone of Central Europe. At present, our model can satisfactorily predict a complex biological process of pseudothecial maturation using only two basic climatic variables, namely mean daily temperature and daily sum of precipitation. The complete adaptation of this model to Polish conditions will use a dataset with observations on pseudothecia maturation of the *L. maculans* – *L. biglobosa* species complex and the atmospheric concentration of their ascospores. This is also one of the first cases where epidemiological modelling will be based upon molecular data (Kaczmarek *et al.*, 2008).

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PCR-detection of fungal pathogens in winter oilseed rape and their survey in the Czech Republic

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Abstract: *Phoma* stem canker caused by the complex of ascomycetous fungi *Leptosphaeria maculans* (Desmaz.) Ces. & De Not. and *Leptosphaeria biglobosa* n. sp. is the most serious disease of oilseed rape in the Czech Republic and worldwide. *Sclerotinia* stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) and premature ripening caused by *Verticillium longisporum* ((Stark) Karapapa, Bainbridge & Heale) are additional damaging diseases of this cruciferous crop. All these pathogens cause specific symptoms and produce fruiting bodies on different parts of plant tissue. Pycnidia are observed in the center of lesions caused by *L. maculans* and *L. biglobosa*. Black sclerotia are formed inside rape stems damaged by *S. sclerotiorum* and abundant amounts of microsclerotia are developing on stems and roots affected by *V. longisporum*. Nevertheless, symptoms especially on stems and roots caused by these fungi are similar and are easily misidentified. Therefore, the objective of our work was the detection of these pathogens in plant tissue and differentiation of *L. maculans* and *L. biglobosa* by polymerase chain reaction using species specific primers. PCR reactions with primer sets described in literature have primarily been optimized using DNA isolated from pure cultures of individual fungal species, which have been determined by morphological and cultivation methods. Fungal DNA has been used as positive control in the following *in planta* tests. DNA was extracted from plant stem tissue and leaf tissue in the case of primary symptoms of *Phoma* stem canker by Gene EluteTM Plant Genomic DNA Miniprep Kit (Sigma) and screened by amplification with primers in pre-optimized temperature conditions. DNA isolation and polymerase reaction was performed firstly on stems with specific symptoms and then on hardly distinguishable symptoms. Some of the described primer sets totally failed in our hands. On the basis of PCR assay, the survey of occurrence of *L. maculans* and *L. biglobosa* in different regions of the Czech Republic was performed during 2007/2008 growing season. In most cases both *L. maculans* and *L. biglobosa* were detected in primary leaf symptoms and stems as well.

Key words: Oilseed rape diseases, Czech Republic, PCR

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Genetic determinism of quantitative resistance to *Leptosphaeria maculans* in WOSR

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Abstract: Stem canker (Blackleg) caused by the fungus *Leptosphaeria maculans* (*Phoma lingam* Tode) is a major disease of *Brassica napus* worldwide causing serious losses on crops in Europe, Australia and North America. The most common and effective way to control this disease is the use of resistant cultivars. Two types of resistance are distinguished. The first type is a qualitative resistance which is considered as specific single-gene resistance and quickly overcome by the pathogen. The second type is a quantitative resistance which is a partial, polygenic resistance mediated by Quantitative Trait Loci (QTL). Polygenic quantitative resistance tends to be regarded as more durable than qualitative resistance because the pathogen would adapt more slowly to it than to a single major resistance gene. Hence, the enhancing sustainability of resistance goes through the exploitation of quantitative resistance. In this pathosystem, the search for quantitative resistance factors has focused on one source of resistance, the variety 'Darmor', for which resistance QTL were detected in two genetic backgrounds.

In this context, we used different methodologies in a complementary way to both identify new QTLs through exploring different genetic backgrounds and improve the accuracy of QTL.

To reduce the confidence interval of QTL, we tested a method using a rational choice of doubled haploid (DH) from 'Darmor-*bzh'* x 'Yudal' population. To identify new QTL and estimate their effects in different genetic backgrounds, we did both a study of association mapping (AM) and a detection of QTL using jointly a collection of winter oilseed rape varieties and connected multiparental populations.

The rational choice of DH helped us to better estimate the confidence interval of some resistance QTL. A collection of 130 oilseed rape varieties characterized for their level of blackleg resistance and structured with 74 markers (SSR, SCAR) allowed us, (i) to choose resistant varieties genetically distant from 'Darmor' in order to create connected population for a future QTL detection; (ii) to do a more exhaustive search of loci associated with resistance by AM. Using this last approach, we showed that marker alleles were associated with stem canker resistance and were mapped (i) in, or close to, the confidence interval of resistance QTL previously identified, (ii) in other regions not harbouring previously detected QTL on the 'Darmor-*bzh'* x 'Yudal' map. This suggested the presence of original quantitative resistance factors within the collection in comparison to 'Darmor'. The identification of resistance QTL within connected populations was in progress. All QTL identified thanks to different approaches would be analyzed and QTL would be compared in terms of position and / or effect and / or precision.

This work proposes an efficient methodology to determine more accurately the structural organization and diversity of resistance QTL in different genetic backgrounds. This study also provides a set of blackleg resistance QTLs as well as elements of choice for the breeders.

Resistance durability of oilseed rape to blackleg assessed in multi-year field experiments

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Abstract: The durability of resistance is an important question in plant disease resistance breeding. To assess resistance durability of oilseed rape to *Leptosphaeria maculans*, we performed several multi-year field experiments, separately for each resistance or combination of resistances. The objective was to simulate in small trials the selection pressure that might be exerted by the resistant lines on fungus populations after their commercial launch. The protocol was based on the recurrent selection of *L. maculans* populations on a resistant line during each growing season, the residues of which were used as primary inoculum the next autumn to contaminate the trial of same multi-year series. This method was used to assess the durability of resistant genotypes in two agricultural regions with distinct agro-climatic characteristics that were distant from each other by several 100 kms (Brittany and central France). Similar dynamics of resistance overcoming was observed. New highly effective resistances to *Leptosphaeria maculans* conditioned by *Rlm6* (MX) and *Rlm10* (LA4+) were introgressed either from *Brassica juncea* to a susceptible *B. napus* genotype or from *B. nigra* into cv. Darmor with quantitative resistance, respectively. Using this protocol the resistance durability of the former was very short *i.e.* 3 years after the first sowing, conversely the latter resistance gene displayed a longer durability of at least 4 years. For assessing the effect of quantitative resistance on the resistance durability of a major gene, *Rlm6* was introduced by backcrosses into two genetic backgrounds, Eurol (susceptible) and Darmor (quantitative resistance). From the nearly isogenic lines without (Eurol, Darmor) and with *Rlm6* (EurolMX, DarmorMX), a multi-year field experiment was conducted for 5 years. Each year, 4 separate field trials were established and each inoculated recurrently with fungal populations selected on 1 of the 4 genotypes. In both trials where Eurol and Darmor residues were used recurrently similar frequency of avirulence/virulence alleles and similar ranking of genotypes for resistance/susceptibility were observed over years. In both trials where residues of MX lines were used in the 2nd year, the size of fungus population dramatically decreased. EurolMX resistance broke down in the 3rd year when the primary inoculum was recurrently selected on EurolMX. In contrast, in the trial where the primary inoculum was the residues of DarmorMX the disease pressure remained low over years and the resistance of DarmorMX was still highly effective in the 5th year of the experiment. These results suggest a slower adaptation and multiplication of the virulent isolates on this latter line and therefore an effect of the polygenic quantitative resistance on the durability of the resistance.

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Are varietal associations of *Brassica napus* a way to manage efficiently specific resistance genes to *Leptosphaeria maculans*?

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Abstract: It is now well established that specific resistance genes to *Leptosphaeria maculans* can be overcome by the pathogen after a few years of commercialization or of recurrent pathogen selection in field experiments when they are introduced into susceptible varieties. We can hypothesize that a decrease of the selection pressure on pathogen populations may allow maintenance of the specific resistance gene efficacy. One way could be to grow varietal associations (mixtures of resistant and susceptible plants) to decrease durably the size of pathogen populations and protect the susceptible plants in the mixture if the pathogen has secondary cycles of multiplication. For assessing the efficacy of a specific resistance gene in varietal associations, we have produced nearly isogenic lines carrying a specific resistance gene, *Rlm6*, introduced from *Brassica juncea* in winter type varieties with ('Darmor') or without ('Eurol') polygenic resistance. We sowed varietal associations with 0, 30, 70 or 100% of seeds with *Rlm6* for both varieties over a three year field experiment and varietal associations with 0, 25, 50 or 75% of *Rlm6* gene for the 'Eurol' variety over two additional years of field experiment. The presence of *Rlm6* gene was checked using a molecular marker specific to the introgression carrying the gene. Leaf spots in autumn and stem canker in spring were scored. We showed that (1) the proportion of resistant plants in plots corresponded well to the initial seed mixtures, (2) the frequency of plants attacked, the average number of leaf spots per plant and the G2 index of stem canker were proportional to the amount of susceptible plants introduced in the mixtures, (3) the isogenic lines on 'Darmor' genetic background are more resistant than the ones on 'Eurol' variety, (4) susceptible plants were less attacked in the mixture containing the highest level of resistant plants depending on the year of experiment. According to these results, experiments are in progress to assess the durability of the efficacy of the resistance gene over years by studying the evolution of avrLm6 virulent gene frequency in the pathogen population in isolated fields contaminated either by pure 'Eurol' or 50% 'Eurol-Rlm6' mixture or pure 'Eurol-Rlm6' residues.

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Are leaf symptoms a way to check an increase of virulent populations on *Rlm7* hybrids?

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Abstract: New genotypes of oilseed rape were recently introduced commercially in France. Their excellent resistance to blackleg (*Leptosphaeria maculans*) is mainly due to a new major resistance gene, *Rlm7*. Such varieties are potentially exposed to a resistance breakdown. The main objective of extension bodies is to promote the durable management of resistance and try to anticipate any possible breakdown. This objective necessitates being able to detect as early as possible the first step of the pathogen population switch towards virulence. Two ideas were developed for such an aim. Firstly, a comparison between leaf symptom density on cultivars having or not having the targeted specific resistance may be used as an indirect indicator of the increase of virulent isolates in the population. The second way was to directly check virulence profiles of the pathogen population. These two methods have been tested since autumn 2004 in the central region of France at fields located between Issoudun and St Florent sur Cher. The first approach appeared to work during the first two experimental years. Nevertheless, epidemics were too low during the last two years to allow us to make a clear conclusion. Virulence profiles were surveyed in this region and also in experimental fields in Versailles, Grignon and Rennes, from isolates sampled from leaf symptoms. The survey showed that *AvrLm7* (avirulent) spores were able to produce leaf symptoms on *Rlm7* varieties. However the leaf lesions were often smaller than those due to virulent isolates, with a huge variability of leaf symptom morphology, even on the same genotype, in the same field, under the same climate. Only a low number of isolates from *Rlm7*-leaf symptoms were identified as *avrLm7* (virulent). These results underline the risk of overestimation of virulence allele frequencies based on foliar symptoms, the need for pedagogic documents and teaching sessions to help experimenter's and local advisors to detect the beginning of resistance breakdown in a bio-vigilant approach, and the difficulties of doing such a survey with limited costs and limited sampling.

Key words: *Leptosphaeria maculans*; *Brassica napus*; virulence; bio-vigilance, resistance durability

Introduction

Genetic resistance of winter oilseed rape (WOSR) to *Leptosphaeria maculans* has been a major target for plant breeders since the beginning of the 90's. The intensive use of the specific resistance gene *Rlm1* in the middle of the 90's induced a prejudicial breakdown (Rouxel *et al.*, 2003). In parallel, a lot of successful efforts have been made to reach high levels of quantitative resistance. Nevertheless, introduction of an efficient specific resistance is the quickest way to reach high levels of resistance and to allow practical control of the disease at the farm level. Recently, new varieties of winter oilseed rape were registered in France and the UK with excellent resistance to *L. maculans*. Evaluation tests based on the gene for gene interaction have shown the introduction of the new *Rlm7* specific resistance in these cultivars. *Rlm7* was firstly introduced in classical or high erucic lines. Since 2004 however, *Rlm7* was mainly introduced commercially in high yielding restored hybrids. After

several years of successful post registration testing these hybrids cover in 2007-08 around 45% of the national WOSR cropping area in France, and locally, where blackleg has been a severe disease, probably around 60% of the market share. Such a success raises questions as to the risk of this specific resistance to be overcome. Since the early 2000's, CETIOM, the French applied research institute for oil crops, advised alternation between groups of cultivars to lower the selection pressure exerted by each resistance gene on the pathogen populations, and thus to slow down the development of virulent isolates (Gladders *et al.*, 2006). In parallel, CETIOM tested in a small production area what could be an appropriate bio-vigilance system for an early detection of the emergence of virulence within the pathogen population. For such a purpose, the simplest approach was to look at leaf spots density in autumn. If the gene for gene interaction worked as defined, and if the population was still mainly avirulent for *Rlm7*, leaf spotting should be low on *Rlm7* genotypes in comparison to genotypes without the efficient specific resistance. A second method was to sample leaf spots and identify virulence profiles of the isolates that were collected. A third method might be to combine these observations with the use of a spatio-temporal model able to predict an increase of virulent isolates in the population (Lô-Pelzer *et al.*, 2008). The present paper presents results from field experiments performed in France during the 2004-2008, where the first two approaches were tested, both in a small scale production area in the "Région Centre" (situated in the central region of France and in different experimental sites where *Rlm7* hybrids were grown.

Material and methods

Locations and designs

Field trials from variety testing networks were randomized blocks designs with 4 replicates. The field trials aimed to compare a set of genotypes sown in elementary plots of 30-50 m^2. The plots include control cultivars and new genotypes under evaluation. In 2006-07, several *Rlm7* restored hybrids were grown. These trials were used to recover isolates from leaf symptoms (Table 1).

The second working area ("Région Centre") was a field production area, located between St Florent sur Cher and Issoudun, over 3 villages around a distance of 250 kilometres south of Paris. The production area was a 10-15 km-long square. In this area, 20 to 30 fields were regularly observed each year, during the cropping season: at the emergence stage, before winter during the second half of November, at the end of winter, at bloom stage, and at the beginning of ripening. The area represented 400-500 ha of WOSR. Fields and sampling area inside the field were geo-tagged using a GPS system. Production of primary inoculum (ascospores in the air) was measured using a Burkard spore trap (Gladders and Musa, 1980).

Cultivars

The main standard varieties used in the pilot area or as controls in the field trials were ES Astrid (*Rlm9*), Aviso (*Rlm9*), Kosto (*Rlm9*), and Grizzly (*Rlm2* and *Rlm3*). The *Rlm7* varieties analysed in this study were (i) Roxet, a line registered in the UK in 2002, and (ii) Ogu INRA restored hybrids from Monsanto registered in France (Exagone, Exocet, Extend) or in the UK (Excel).

During the first season (2004-2005) only few fields were sown with Roxet variety in the studied area. In the following years, the acreage cropped with *Rlm7* restored hybrids increased dramatically due to their good evaluation in variety testing networks: around 5% in 2005-06, 25% in 2006-07, 40% in 2007-08, and around 60% in 2008-2009.

Table 1. Characterization of isolates recovered from *Rlm7* genotypes in field variety testing trials in 2006-07.

Site	**Administrative Department**	**Plant genotype sampled**	**Number of isolates recovered**	**% of *L. maculans***	**Number of virulent isolates *(avrLm7)***
Grignon	78	Exagone	35	100 %	14
Versailles	78	Exagone	37	95 %	0
Bagneux	36	Extend	47	98%	2
Civray	18	Exocet	43	98%	7
Francillon	36	Exagone	32	100%	3
Le Blanc	36	Exagone	35	83 %	1
Senné	86	Exagone	35	77%	1
Grignon	78	Exel, Roxet	80	5%	0
Total		**5 genotypes**	**344**	**73 %**	**28 (8%)**

Field observations

Leaf spots in autumn were recorded in November in the different fields, on 4 random samples of 25 plants for each field or plot. The final disease index G2 was determined from the observation of 8 samples of 5 plants (Pierre and Regnault, 1982; Aubertot *et al.*, 2004).

Agronomic practices were recorded by questioning farmers. Sampling methods and agronomic observations applied were those recommended in CETIOM's WOSR experimentation guide (2005) (plant density, plant fresh weight, flowering type, height, yield components, grain yield).

Pathogen isolation and characterization

20 leaves per field, with at least one leaf spot per leaf, were collected in November for immediate pathogen isolation. Before isolation itself, a numeric picture of the leaf spot used for isolation was taken. Single-pycnidium isolates were collected from one lesion per affected leaf (West *et al*., 2002). A PCR procedure was used to distinguish *L. maculans* from *L. biglobosa* before plant tests (Balesdent *et al.*, 1998).Virulence profiles of *L. maculans* isolates were identified using a cotyledon inoculation test according to Balesdent *et al.* (2006). Westar (no *Rlm*) or Goeland (*Rlm9*) were used as control genotypes.

Results

Since the rainy years 1999 and 2000, autumn was dry from 2001, especially in September. Spores traps showed only small amounts of ascospores, occurring relatively late in the season, i.e. never before the beginning of October (data not shown). This was the case for the four autumn seasons that occurred during the study (2004-2007). Significant levels of leaf spots were not observed before November in autumn 2004 and 2005 (growing seasons 2004-05 and 2005-06). During the next two years, levels of leaf spots were particularly low (less than $20/m^2$), even for classical varieties without efficient specific resistance genes.

For the first two years of observations, leaf spot densities ranged from 0 to more than 250 spots/m^2 for classical varieties without effective major genes (Figure 1). For fields cropped with *Rlm7* genotypes, leaf spot densities were below a threshold of 50 spots/m^2, whenever the fresh biomass was less than 2500 g/m^2. Two exceptions were observed for fields under organic management with very early sowings (late July) and fields with large amounts of organic fertilization applied before sowing. In such situations leaf areas were particularly large with high N content.

Virulence profiles of isolates recovered from genotypes without effective specific resistance during autumn 2004 were similar to that described previously in France (Balesdent *et al.*, 2006), with 0% of *avrLm7* isolates, 92% of *avrLm1*, 100% of *avrLm3* and *avrLm9*, and 83% of *avrLm4*. All these isolates belonged to the *L. maculans* species (Table 2 and data not shown).

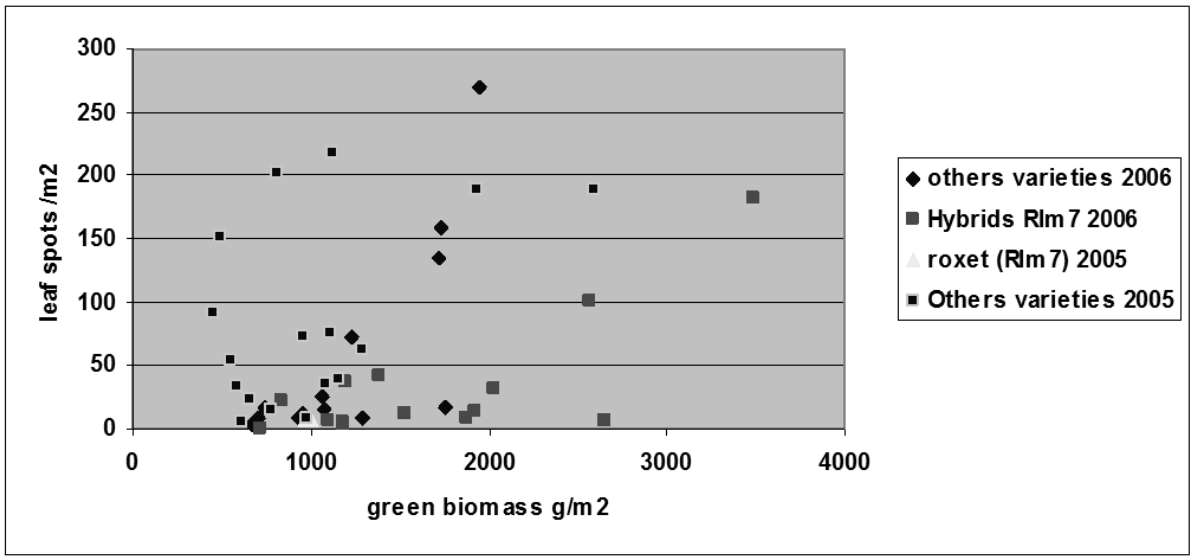

Figure 1. Relationship between fresh biomass per m^2 before winter and number of blackleg leaf spots per m^2 in the pilot production area during seasons 2004-05 and 2005-06. Each dot corresponds to the mean values measured for one field. Pale squares and triangles correspond to fields cropped with *Rlm7* genotypes and small, dark squares and diamonds to fields cropped with varieties without effective specific resistance

On *Rlm7* genotypes, the proportion of *L. maculans* versus *L. biglobosa* was year dependant. The proportion of *L. biglobosa* was higher for the last two years, which were also characterized by lower levels of infection by *L. maculans*. A high percentage of *L. maculans* isolates were unexpectedly identified as *AvrLm7* from cotyledon tests, which looked contradictory to the gene-for-gene interaction theory. The proportion of *AvrLm7* isolates was stable over years (average value 94%). However the leaf lesions from which these isolates originated were often smaller than those due to virulent isolates and were surrounded by a dark margin. Moreover a huge variability of leaf spots morphology was observed, even on the same genotype, in the same field under, the same climate.

Table 2. Number and characterization of isolates recovered from production WOSR fields of the "Région Centre" pilot area during four successive cropping seasons (September 2004 to July 2008).

Growing season	**Isolates recovered from***	***L. biglobosa***	***L. maculans***		
		Total	***Total***	***Virulent isolates avrLm7***	***Avirulent isolate Avrlm7***
2004-2005	*Rlm7* genotype (Roxet)	2	19	3	16
	Others	0	163	0	163
2005-2006	*Rlm7* genotypes (Exagone, Exocet)	7	35	5	30
	Others	0	17	0	17
2006-2007	*Rlm7* genotypes (Exagone, Exocet, Extend)	25	135	7	128
	Others	0	14	0	14
2007-2008	*Rlm7* genotypes (Exagone, Exocet, Extend)	129	80	2	78
	Others	0	67	0	67

In the collection of isolates from *Rlm7* genotypes grown in the variety testing field trials (Table 1), the proportion of *L. biglobosa* was also dependant on the site, from 0 to 95%. The two extreme values were observed from neighbouring sites in Grignon. As for the "Région Centre" pilot area, isolates from leaf spots on *Rlm7* genotypes were mainly identify as *AvrLm7*, with an average percentage of 92% *AvrLm7* isolates. Similar results were found at field trial sites near Rennes (Data not shown).

Discussion

French WOSR producers experienced the breakdown of the *Rlm1* specific resistance at the end of the 90's, following its widespread use (Rouxel *et al.*, 2003). Fungal populations were subsequently characterized in different sites in autumns 2000 and 2001, and results underlined at all the sites a clear predominance of virulent *avrLm1* isolates, especially from sites in "Region Centre" (Balesdent *et al.*, 2006). Our results during autumn 2004 are in accordance with the data from 2000-01. Four years later, virulence profiles were similar, with residual populations which were *AvrLm1* (8%) or *AvrLm4* (17%). Even with the dramatic commercial success of *Rlm7* hybrids over several years, populations recovered from classical varieties were exclusively *AvrLm7* and only a few *avrLm7* individuals were identified on *Rlm7* genotypes, which is in accordance with the lack of sign of breakdown observed to date (final G2 index was always under 1 for *Rlm7* hybrids). Nevertheless, some virulent *avrLm7* isolates were recovered from *Rlm7* genotypes. Under selection pressure by *Rlm7* genotypes, these

isolates were expected to become more numerous and to overcome the specific resistance. In front of the risk of breakdown, the purpose of this study was to test methods to monitor virulent *L. maculans* populations and to detect as soon as possible the beginning of the breakdown.

The first method tested was to look at leaf spot density on varieties with or without the effective specific resistance. Following the gene-for-gene interaction theory, a genotype with an effective specific resistance would be expected to have fewer symptoms. If the leaf spots density on *Rlm7* varieties was becoming closer to that seen on classical genotypes, this would suggest indirectly, that the fungus population was becoming virulent. Our results suggest that such a criterion would work when the ascospore release was sufficient (Figure 1). In autumn 2006 and 2007 however, ascospore releases were so low that differences between genotypes were not visible, due to very low levels of leaf spots, whatever the variety (<10 leaf spots/m^2).

The main interest of such a bio-vigilance method was its simplicity, nevertheless there are several difficulties. The first difficulty was that symptom density on leaves was very dependent on the climatic and epidemiologic scenario of that year. The proposed threshold was established only with 20 to 30 fields over 2 years. The two following years, with less ascospores release we have already seen that this threshold was not appropriate. There was a second difficulty. When WOSR was introduced long-term in crop rotations, there was often a high level of volunteers, sometimes more than the drilling density. Oilseed rape seeds are known to be able to survive for a long time in the soil. If the proportion of volunteers from a non *Rlm7* variety is important, then the leaf spot density estimation will be overestimated due to the problem of distinguishing resistant plants from susceptible volunteers. A third difficulty was the ability to identify leaf symptoms properly. Typical *L. maculans* leaf spots are grey with a black punctuation from pycnidia. However the analysis of a high number of photos revealed that *L. maculans* spots on *Rlm7* genotypes could be confused with *L. biglobosa*, but sometimes also with *Pseudocercosporella*, or *Alternaria* leaf spots. The collection of leaf spots pictures and the matching isolate characterization established here will now allow us to perform training sessions with illustrated documents and to prepare a computer didactical programme available on a CD or via the web.

Another way to check the occurrence of virulent sub-population was through leaf sampling and fungal isolation from leaf spots. In such an approach, the limits are sampling size and the ability to further characterize isolates, for example, isolation and subsequent virulence identification through cotyledon tests are time consuming procedures. Molecular markers are available to differentiate *L. maculans* and *L. biglobosa* (Balesdent *et al.*, 1998), and to identify virulences *avrLm1* and *avrLm4* (Gout *et al.*, 2006, Parlange *et al.*, 2009). These tools save time, especially because they can allow virulence typing directly from the leaf symptom, without fungal isolation. Nevertheless molecular markers to detect *avrLm7* are not yet available. Therefore, since 2004, we isolate and characterize 200 strains per season, which is rather low for the target. Only availability of markers for all the virulences of interest, will allow us to perform a reasonable level of sampling and to accurately estimate virulence frequencies.

Samples taken each year, since 2004 have shown different proportions between the two *L. maculans* and *L. biglobosa* species. The proportion of *L. biglobosa* was higher when autumns were dry with poor and late ascospore release. This result was in accordance with previous studies (Penaud and Pinochet, unpublished data). The main original result from our work was to find a majority of avirulent isolates from leaves of *Rlm7* genotypes (92-94%), which was not in accordance with the gene-for-gene theory. This result, firstly found on the pilot production area from "Région Centre", was confirmed in 2006 at a multilocal scale with roughly equivalent percentages. A first hypothesis to explain this result could be that *AvrLm7*

isolates may produce leaf spots on *Rlm7* genotypes under particular environmental conditions or plant growth stage, which may differ between field conditions and the controlled conditions used in cotyledon test. Huang *et al.* (2006) showed similarly that the *Rlm6*/*AvrLm6* interaction was dependant on temperature and the wetness duration. Secondly, we have seen that final severity ratings (G2 index) were good for *Rlm7* genotypes (low levels of index G2). It could therefore also be hypothesized that, although being able to initiate leaf symptoms, *AvrLm7* isolates are later on restricted to the leaf spot and unable to grow systemically to develop stem canker. This hypothesis is consistent with the frequent observation that *AvrLm7* isolates produce, on *Rlm7* plants, small leaf spots surrounded by dark margin, indicative of the resistance response of the plant. The percentage of avirulent isolates able to grow down to the stem base in *Rlm7* genotypes still needs to be measured, in order to evaluate the possibilities of recombination between avirulent and virulent isolates, which could increase the speed of dispersal of virulent isolates but also contribute to the survival of *AvrLm7* isolates in the population.

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Occurrence of different *Phoma lingam* pathotypes on oilseed rape cultivars with different resistance gene background

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Abstract: In the frame of a field study related to the artificial improvement of *Phoma* disease pressure, we monitored the disease incidence and severity of *Phoma lingam* on a small set of cultivars at a location close to Göttingen, central Germany. Visual assessments were performed on leaves, stems and the root collar. Furthermore, isolations were taken from diseased tissues. Isolates were differentiated into pathotypes by the use of the cotyledon test using the differential set Lirabon, Quinta, Glacier, Jet Neuf, Doublol (all of them *rlm7*) and Caiman (*Rlm7*)**.**

High disease incidences but moderate disease severities were recorded. Inoculated variants (conidia spraying, stubble deposition) showed no significant differences to controls. Control of *Phoma* stem canker by intense spraying of ERIA (ai: Difenoconazol, intervals of about 3 weeks) was always very effective and highly significant in all cultivars. Diseases severities displayed the superior field resistance of the *Rlm7* cultivar Caiman compared to the other *rlm7*-cultivars tested.

Phoma isolations were performed from cultivars Caiman and Oase. A number of 279 single pycnidiospore and hyphal tip isolates were checked in a first screen on cotyledons of cv. Lirabon to differentiate *L. maculans* (virulent on cv. Lirabon) and *L. biglobosa* (avirulent on cv. Lirabon). About 52 % of isolates were assigned to *L. maculans.* Significantly more *L. maculans* isolates originated from cv. Oase. *L. biglobosa* was predominant on cv. Caiman (about 96%). Race differentiation showed that diversity of *Phoma*-pathotypes was higher on cv. Oase than Caiman. Among the isolates originated from cv. Oase there were 9 *avr*7-isolates (3.3%) showing the presence *avr7* isolates in central Germany without a history of cultivating *Rlm7* varieties.

Key words: *Phoma lingam*, oilseed rape, resistance gene, avirulence gene, *Rlm*7

Introduction

Phoma lingam is the causal agent of stem canker of oilseed rape. This disease is the most economically important threat to oilseed rape production worldwide. Early studies reported different pathotypes of this pathogen (e.g. Cunningham, 1927). Epidemics were related to the occurrence and frequency of aggressive forms of this fungus, which now is believed to belong to the species *Leptosphaeria maculans*, whereas less damaging pathotypes are assigned to the species *L. biglobosa*. The huge damage related to *L. maculans* epidemics lead to breeding programmes intended to screen for new resistance sources and the introgression of new resistance genes. Nowadays, there are numerous major resistance genes reported (*Rlm*1-9 and others), which are used for the development of commercial cultivars. Comparably new are *Rlm*7-cultivars, which possess a resistance gene with broad efficacy against all German *Phoma* pathotypes known so far. This resistance also performs very effectively under German growing conditions. The aim of this study was to monitor the occurrence of *avr*7-isolates (virulent on *Rlm*7-genotypes) on both *Rlm*7 and *rlm*7 cultivars with respect to the durability of this resistance.

Material and methods

Field experiment

In autum 2007, four cultivars (Oase, Toccata, Cooper and Caiman) were sown at a field site (Dragoneranger) close to Göttingen. The experimental design was a randomized block design. The plot sizes were 36 m^2 divided in equal subplots for plant sampling and yield assessment, respectively. The experiment comprised the above mentioned 4 cultivars, 4 treatments and 4 repetitions. The treatments were: (i) control (natural infection), (ii) inoculation at BBCH 16 by spraying a spore suspension (mixture of 3 *L. maculans* isolates at a rate of 100 ml/m^2, using a spore concentration of $1*10^6$/ml), (iii) deposition of oilseed rape stubbles covered with pseudothecia (13.3 g/m^2) at BBCH 16 and (iv) fungicide control (application of Eria, *c.* every third week). In order to accomplish a higher infestation rate all inoculated plots were rolled using a Cambridge roller. Ratings were done in October 2007, May and July 2008 by counting numbers of *Phoma*-lesions and /or rating according to Krüger (1982) for root collar and stem symptoms. In October and May the length, extent and depth of root collar lesions were assessed in detail and results calculated as "volume of diseased tissue" (VDT) according to Kutcher *et al*., (1993) with slight modifications (scale of 0-9 instead 0-4). Yields of the variants couldn't be recorded due to a severe hailstorm.

Isolation procedure

At all three rating time-points (in October and May leaf-lesions, in July lesions of stem and root collar) isolates were gathered using natural infected plants of cv. Oase (*rlm*7) and Caiman (*Rlm*7). Therefore leaf-lesions were cut out, placed in moisture chambers and incubated at least for three days. After incubation spores were discharged from pycnidia, which were transferred to V8-media. Lesions of stems and root collars were cut out, surface sterilized, cut diagonally and incubated in moisture chambers for three to four days. Sprouting mycelium was then transferred to V8-media and after another incubation of roughly one week, hyphal tips of all isolates were transferred to new V8-media. Incubation was always done at room temperature under natural light conditions.

Production of spore suspensions

For pathotyping of isolates spore suspensions were produced. Production of pycnidia was initiated by scratching mycelium covered agar plugs on V8-media. During two to three weeks, lots of pycnidia were produced. Pycnidiospores were harvested by flooding the surface with 5 ml of sterile water. Spores were suspended with the help of a microscopic slide and filtered. Spore density was adjusted using a haemocytometer to 10^7 spores/ml. Aliquots of the suspensions were stored in 1.5 ml Eppendorf reaction vials at -20 °C.

Characterization of isolates

All isolates were first inoculated on 7-day-old oilseed rape seedlings of cv. Lirabon to distinguish between *L. maculans* and *L. biglobosa.* Cotyledons were punctured on each half with a needle and 10 µl of a spore suspension was pipetted on the wounds. Rating was done 14 days post inoculation when *L. maculans* isolates showed huge lesions with tissue collapse and mostly intense sporulation, whereas *L. biglobosa* only shows small lesions due to hypersensitive response without any sporulation. Those isolates assigned to be *L. maculans* were than inoculated on the differential set comprising the cvs. Lirabon (as control), Quinta, Doublol, Jet Neuf, Glacier and Caiman. Assessments were performed 14 dpi using the IMASCORE rating scale (Volke, 1999).

Statistical analysis
Statistical analyses were performed using the software XL-Stat Pro 7.5 (Addinsoft, Andernach, Germany). Analysis of variance was calculated followed by comparison of means for significant differences using the Tukey test on a level of $P \leq 5\%$.

Results and discussion

Investigation of different inoculation methods
Phoma pressure was moderate in the growing season 2007/208. Leaf spotting in autumn showed mean lesions numbers ranging between 4-8 lesions at growth stage BBCH22-24 on the more *Phoma* susceptible cultivars Oase and Tocatta (Figure 1). The *Phoma* disease pressure is probably not well presented by this sampling date, because main ascospore discharge started beginning of October recorded by a Burkhard spore trap (mean of ca. 20 spores a day, max. 120, data not shown). However, differential reactions of cultivars match the categorization of the "German National Descriptive List of Varieties". Caiman considered to be very resistant showed only very few lesions in the control treatment (mean < 1), whereas Oase and Tocatta considered to be more sensitive showed higher mean numbers of lesions (mean of 7 and 5, respectively). The variety Cooper ranged in-between (mean of 2). The spore spraying and stubble deposition treatments showed no differences to the control. The recorded data show a very effective control of *Phoma* leaf spotting.

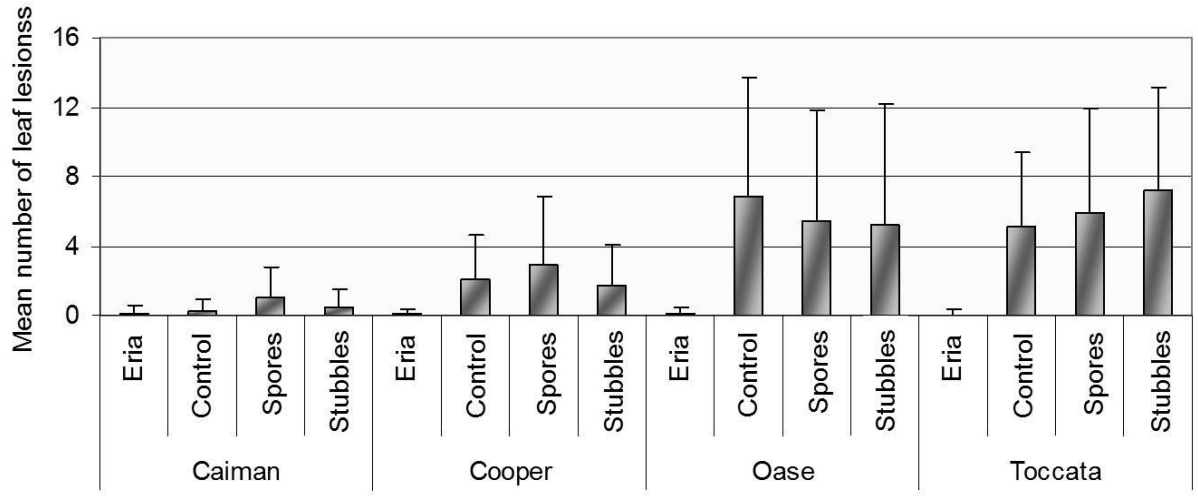

Figure 1. *Phoma lingam* leaf spotting in autumn at growth stage BBCH 22-24 of cvs. Caiman (*Rlm7*), Cooper, Oase and Toccata (all *rlm7*) of variants ERIA-controls, natural infection (control), spore inoculation (spraying of 10^6 spores/ml at BBCH 16) and stubble inoculation (inoculation at BBCH 16), n = 120

The moderate disease pressure during autumn was also translated in moderate disease expression in growth stage BBCH 89 (Figures 2 and 3.). The disease incidences ranged, depending on the cultivar being considered, between 80-95% on stems and 45 and more than 95% on root collars in control plots which represents natural infection (Figure 2). Our field trails did not show any significant differences at this late stage between natural infection and

artificial inoculations performed with either spraying spores or depositing stubbles. This was in accordance to experiments from previous years, where only stubble deposition showed a slight tendency to increase the disease pressure. Spray inoculations with pycnidiospores did neither this nor in the years before enhance the disease pressure significantly, even when plants were mechanical injured by the use of a Cambridge roller.

The ERIA treatment can be considered to be a very effective control measure for *P. lingam* throughout the experimental season if regularly applied. This was most obvious and also statistically significant on the basis of the disease severity parameter VDT (refer to Figure 4). Again, at the later growth stage BBCH 89 disease severity displays the superior field resistance of cultivar Caiman (*Rlm7*) compared to the other *rlm7*-cultivars tested. *Rlm7* field resistance was as effective as the intense chemical control achieved by ERIA applications, as the statistical analysis confirmed. *Phoma* susceptibility ranking of cultivars was consistent with the "German National Descriptive List of Varieties". Oase and Toccata were classified as the most *Phoma* susceptible varieties within the studied set of cultivars. However, Cooper is classified to be more resistant and Caiman to be the most resistant cultivar.

Yields of the different treatments planned to be surveyed was not recorded due to a hailstorm two weeks before harvest leading to total loss of the crop.

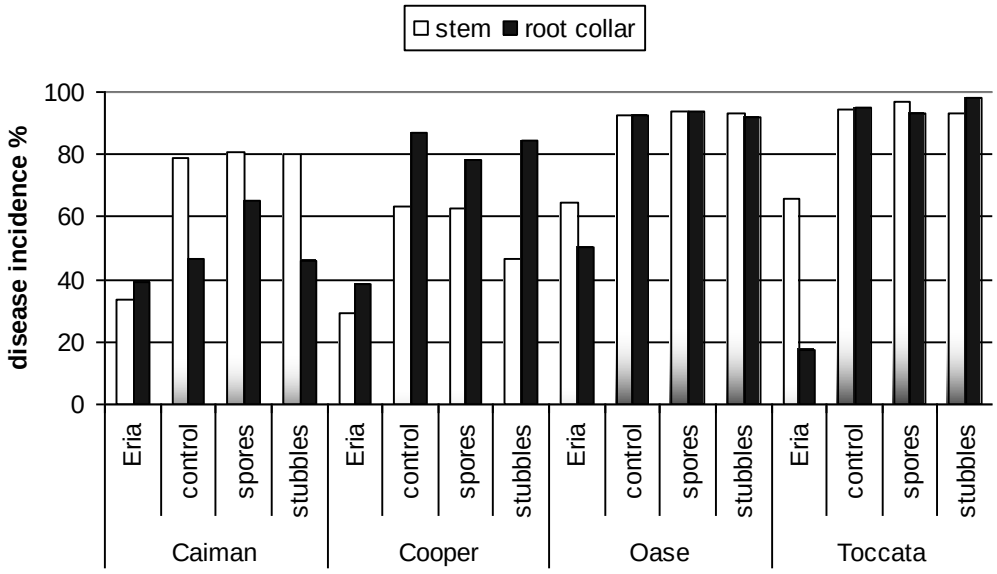

Figure 2. *Phoma lingam* disease incidence at growth stage BBCH 89 on stems and root collars of cvs. Caiman (*Rlm7*), Cooper, Oase and Toccata (all *rlm7*) of variants ERIA-controls, natural infection (control), spore inoculation (spraying of 10^6 spores/ml at BBCH 16) and stubble inoculation (inoculation at BBCH 16), n = 120

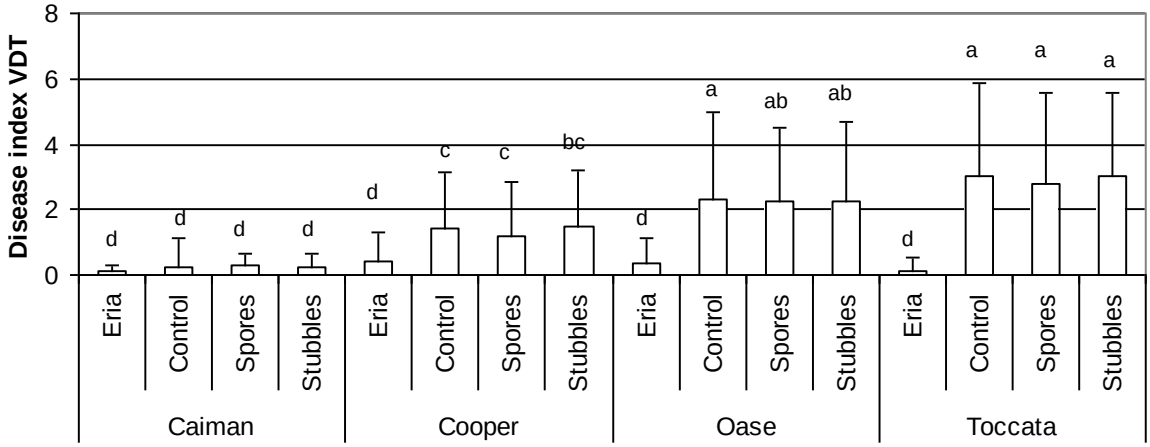

Figure 4. *Phoma lingam* disease severity (VDT = "volume of diseased tissue") at growth stage BBCH 89 on root collars of cvs. Caiman (*Rlm7*), Cooper, Oase and Toccata (all *rlm7*) of variants ERIA-fungicide control, control (natural infection), spore inoculation (spraying of 10^6 spores/ml at BBCH 16) and stubble inoculation (inoculation at BBCH 16), n = 120. Tukey-test (P 0.05).

Differentiation of L. maculans and L. biglobosa

Isolations were performed at three different time points from cultivars Caiman and Oase. A number of 279 single pycnidiospore and hyphal tip isolates were checked in a first screen on cotyledons of cv. Lirabon to differentiate *L. maculans* (virulent on cv. Lirabon) and *L. biglobosa* (avirulent on cv. Lirabon).

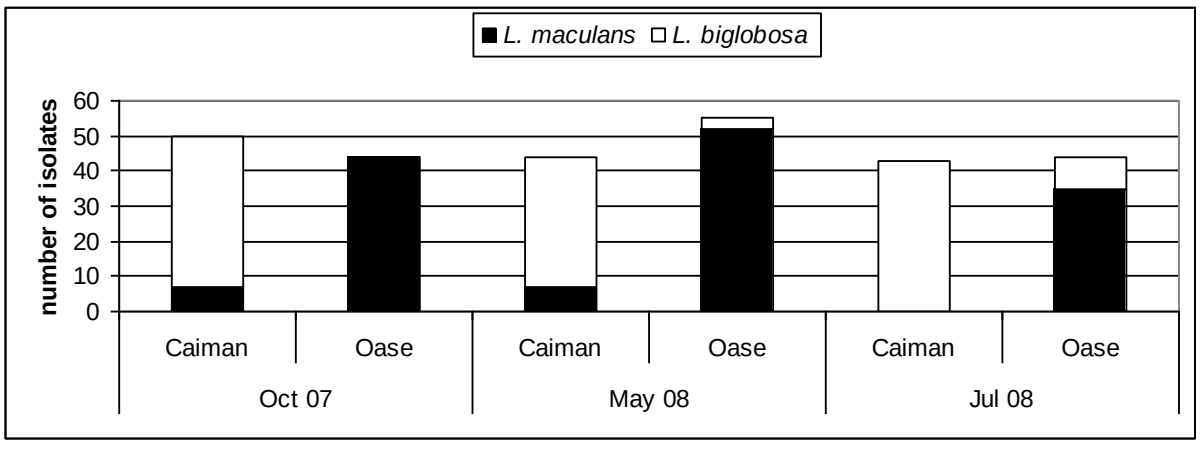

Figure 5. Species differentiation of isolates collected at different time points (Oct 07 – leaf lesions, May 08 – leaf lesions, July 08 – stem lesions) originated from cvs. Caiman (*Rlm7*) and Oase (*rlm7*) via the cotyledon test using differential reactions of cv. Lirabon.

About 52% of isolates were assigned to *L. maculans.* Significantly more *L. maculans* isolates originated from cv. Oase. *L. biglobosa* was predominant on cv. Caiman (about 96%) (Figure 5).

Race differentiation of 115 *L. maculans* isolates mainly originating from *Phoma* lesions of true leafs (107 of 115) and mainly originating from cv. Oase (ca. 90%) showed that diversity of *Phoma*-pathotypes was higher on cv. Oase than Caiman (Figure 6). Isolates of race A1 were dominant on Oase (65%, n = 67) as well as on Caiman (92%, n = 11). A further race detected on Caiman was A2 (ca. 8%, n = 1), whereas races A2 (ca. 12%, n = 12), A3 (ca. 3%, n = 3) and A5 (ca. 14%, n = 14) were detected in leaf and stem lesions of cv. Oase. Among the 103 isolates originating from cv. Oase there were 9 *avr*7-isolates, which are assumed to have overcome the resistance of Caiman. In preliminary experiments, a subset of these isolates does not show high virulence on either true leaves or hypocotyls of this variety (data not shown). These results may indicate that adult resistance of cv. Caiman is not only due to the major resistance *Rlm7*. Despite these preliminary results our findings show that virulent isolates are already among the *L. maculans* population without any preselection by intense cropping of *Rlm7* cultivars. The proportion of about 3.3% (7% considering only *L. maculans*) of the studied isolates shows that *Rlm7* is at risk to be overcome.

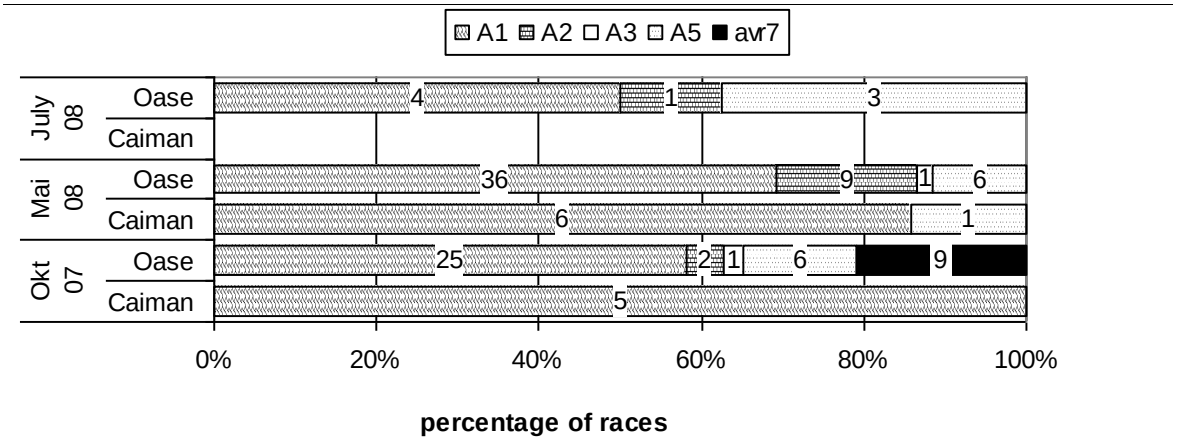

Figure 6. Race differentiation of *L. maculans* isolates collected at different time points (Oct 07 – leaf lesions, May 08 – leaf lesions, July 08 – stem lesions) from cvs. Caiman (*Rlm7*) and Oase (*rlm7*). Numbers of isolates are indicated in the respective bars.

Conclusions

L. maculans avr7-isolates were isolated in a frequency of 3.3% at our experimental site in Central Germany. Although *avr7* isolates were detected, field resistance of cv. Caiman was superior to other varieties. Isolates originated from cv. Caiman mainly proved to be *L. biglobosa*. Although cv. Caiman possess *Rlm7*, *Avr7*-isolates were isolated from leaf and stem lesions. Due to the presence of *avr7*-isolates, there is a further need to monitor the *Rlm7*-adaptation of the *L. maculans* population with respect to the durability of *Rlm7*.

Acknowledgements

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Avirulence genes of *Leptosphaeria maculans*: diversity of mechanisms to become virulent and perspectives

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Abstract: *Leptosphaeria maculans*, a dothideomycete fungus causing stem canker on oilseed rape, develops gene-for-gene interactions with its host plants, oilseed rape (*Brassica napus*) and related species (*B. rapa*; *B. juncea*...). Such interactions imply direct or indirect recognition of avirulence gene products ('AvrLm', for Avirulence gene of *L. maculans*) by resistance genes ('Rlm' for Resistance to *L. maculans*). Recently, we have cloned three *AvrLm* genes, *AvrLm1*, *AvrLm6* and *AvrLm4-7*, which was a starting point to analyse the molecular events responsible for the loss of avirulence function when the fungal populations are faced with the deployment of novel *Rlm* genes. All three *AvrLm* genes were found to be located in a peculiar genomic context, i.e. solo genes within large non-coding, heterochromatin-like regions rich in truncated and inactivated repeats. Such a genomic environment probably conditions evolution mechanisms towards virulence, as the two main events identified were either large deletions of a chromosomal segment spanning the avirulence gene, or inactivation of the gene by RIP ('Repeat Induced Point') mutations. RIP is a mechanism of gene inactivation usually directed toward duplicated sequences in fungal genomes, and it is suggested that the location of the Avr genes in repeat-rich region may led to frequent RIP inactivation of Avr genes in *L. maculans*. However, other mechanisms of inactivation may also be found, including insertion of repeated sequences in the gene, small-scale deletions or even single-base mutations. The diversity of these mechanisms, along with the sexual mating occurring each year in the life cycle of the fungus, may explain how rapidly *L. maculans* populations adapt to selection pressure exerted by cultivars harbouring novel resistance genes.

Characterization of specific resistances to *Leptosphaeria maculans* in recent winter oilseed rape (*Brassica napus L.*) commercial varieties

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Abstract: Blackleg is actually, with *Sclerotinia*, the main disease worldwide for oilseed rape. Its impact on the production level has supported a lot of research efforts in Canada, Australia, and Europe in the fields of agronomy, plant pathology and plant breeding. Among the ways to control the disease at the production level, the use of tolerant varieties is the more developed. Breeders have introduced quantitative as well as specific resistance. Nevertheless, in the recent years break downs of resistance have occurred in several places. In France, the widely used *Rlm*1 was overcome in the late 90's. A similar figure occurred in Australia a few years later with the *Sylvestris* specific resistance introduced in Surpass 400, a popular variety. Compared to a long and difficult process needed to breed quantitative resistance genetic factors, the introduction of known specific resistances looks easier and quicker for breeders, and efficient for users. Characterization of new specific resistances has been very active in recent years in *Brassica napus* and in its parental species. Nevertheless, controlled introductions with backcrosses and marker assisted breeding are not so easy, mainly due to clustering of major genes on the genome. For extension bodies it has become a main objective to be able to identify such resistance in commercial varieties, in addition of traditional evaluation of the general disease susceptibility. This information is needed to develop and promote durable management strategies of such resistances. In Europe several extension bodies like CETIOM in France have started advising farmers for durable management of resistance. In France, in connexion with INRA, commercial varieties characterization has been underway since 2002. After 6 years, and with more than 150 of the new varieties classified, this paper aims to describe the results and to explain how resistances are associated in commercial varieties.

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A duplex PCR to follow the frequencies of avirulence 1 and 4 alleles in field populations of *Leptosphaeria maculans*

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Abstract: *Phoma* stem canker (blackleg), caused by *Leptosphaeria maculans*, is one of the most damaging diseases of oilseed rape worldwide. *L. maculans* exhibits gene-for-gene interactions with its host plant, where fungal avirulence (*AvrLm*) genes are the counterparts of plant resistance (*Rlm*) genes. The pathogen is able to rapidly adapt to the selection pressure exerted by a novel resistance gene. In France for instance, the large-scale cropping of *Rlm1* cultivars was followed by the loss of efficiency of this resistance gene within a few years, as the virulent allele at the *AvrLm1* locus became prevalent in the fungal population. Methods to follow the avirulence alleles frequencies in the pathogen populations are therefore needed to allow a more durable use of available resistance genes. The duplex PCR method described here makes it possible to jointly identify the alleles at the *AvrLm1* and *AvrLm4* loci in *L. maculans* isolates which are avirulent at the *AvrLm7* locus (*i.e.* nearly all the isolates, since the avirulence at this locus is highly prevalent at the moment). Based on two simplex PCR methods of INRA BIOGER (Versailles, France), the method was further simplified since no DNA extraction is needed. It is therefore much less time-consuming than cotyledon tests. The method was validated on 200 isolates that had previously been characterised by cotyledon tests: 100 isolates were avirulent at the *AvrLm1* and *AvrLm7* loci and virulent at the *AvrLm4* locus, and 100 were avirulent at the *AvrLm4* and *AvrLm7* loci and virulent at the *AvrLm1* locus. The results of the PCR test were in accordance with those expected with 99.5 and 99 % of the cases for the *AvrLm1* and *AvrLm4* loci being identified, respectively. The next step of the work will be to improve the method so that isolates that are virulent at the *AvrLm7* locus can also be analysed.

Key words: avirulence gene, blackleg disease, durability, *Leptosphaeria maculans*, resistance

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Elicitors of *Leptosphaeria maculans* inducing resistance to blackleg in oilseed rape

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Abstract: Compounds produced by *L. maculans* into various cultivation media were tested for their ability to induce defence gene expression by means of RT-qPCR and SAR by inoculation test. After removing toxins and other low molecular metabolites by dialysis, the medium was concentrated and subsequently subjected to fractionation by ionex chromatography. Separated fractions inducing *PR1* expression were digested by trypsin. Tryptic hydrolysates lost their *PR1*-inducing activity, which indicates a protein nature of the efficient elicitors.

Key words: induced resistance, oilseed rape, elicitors, *Leptosphaeria maculans*

Introduction

Leptosphaeria maculans, an ascomycetous fungus, is a causal agent of one of the most devastating diseases of oilseed rape worldwide, *Phoma* stem canker (Howlett *et al.*, 2001). Control of this disease is usually achieved by fungicides and utilization of resistant cultivars. However, single gene based resistance is easily broken owing to high evolutionary potential of this pathogen, which makes searching for alternative crop protection strategies, including induced resistance, highly desirable (Walters *et al.*, 2005). With respect to its hemibiotrophic lifestyle, induction of resistance to this pathogen is more complicated than to biotrophs and a number of inducers fail to induce resistance.

The aims of our study were to separate and partly characterize elicitors produced by *L. maculans* and secreted into a liquid cultivation media, and to test their ability to induce resistance against *L. maculans* infection in oilseed rape.

Material and methods

Plant material and treatment

Oilseed rape (*Brassica napus*) plants of cv. Columbus were cultivated hydroponically in a half strength Steiner's solution in a cultivation room set as follows: temperature day/night 22 °C/18 °C; photoperiod 16 h/8 h. Two week old plants were sprayed with chemical inducers: 30 μM benzothiadiazole (BTH), 3 mM salicylic acid (SA), 10 mM β-aminobutyric acid (BABA), 1 mM riboflavine, 200 μM menadione sodium bisulphate (MSB) and elicitors from cultivation media of *L. maculans*.

***Cultivation of* Leptosphaeria maculans**
100 ml media were inoculated with 10^7 spores of *L. maculans* JN2 or JN3 and cultivated one or two weeks alternatively in the dark at 26 °C, 100 rpm. Following media were used: Fries, potato-dextrose, creatine sucrose, potato-carrot, Gamborg B5 and modified Gamborg B5.

Preparation of elicitor fractions
Medium was separated by filtration and dialysed 3 times against distilled water (20 fold volume of sample) in the dialysis tubing with cut-off of 8 kDa. Elicitors were further separated by adsorption on annex (HiTrap Q-Sepharose FF) or catex (HiTrap CM-Sepharose) columns. Active fractions were digested by trypsin (trypsin:protein ratio 1:90).

Biological assay and gene expression
Cotyledons of *B. napus* plants were inoculated with conidial suspension (10^7/ml) 72 h after treatment with inducers or elicitors. Area of lesions was quantified two weeks after inoculation by image analysis using Dplan4Lab software. Expression of *PR1* (U21849) was followed by qPCR 24h after the elicitor treatment.

Results and discussion

***Resistance-inducing effect against* L. maculans**
Both chemical SAR inducers and elicitors produced by *L. maculans* to the cultivation medium (Fries) were tested for their ability to induced resistance against this pathogen in oilseed rape cv. Columbus. Cultivation medium Fries strongly decreased lesion size induced by *L. maculans* infection. The level of protection was even higher than that induced by BTH. Other compounds under study did not display such resistance-inducing effect, moreover, MSB, which was previously demonstrated as an efficient inducer of resistance against *L. maculans* (Borges *et al.*, 2003) did not prove these results (Figure 1).

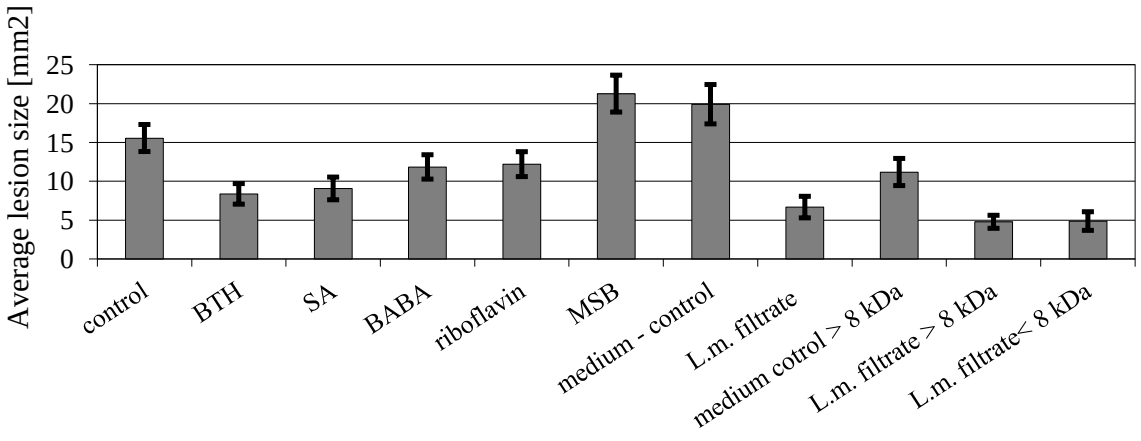


Figure 1. Evaluation of induced resistance based on symptom development on cotyledons. Chemical resistance inducers: BTH 30 μM, SA 3 mM, BABA 10 mM, riboflavin 1 mM, menadione sodium bisulphite (MSB) 200 μM and *L. maculans* medium fractions obtained by dialysis.

To characterize efficient elicitor fractions, cultivation medium was dialyzed to separate low molecular metabolites (< 8 kDa). Both, low and high molecular fractions induced resistance against *L. maculans.*

Elicitors from L. maculans induce PR1 expression

L.maculans cultivation medium (Fries) induced *PR1* expression 24h after the application on *B. napus* cotyledons (Figure 2). Fractions bound either to catex or annex columns were tested for their ability to induce *PR1* expression. The active fractions were subjected to digestion by trypsin. Loss of the activity of tryptic digests indicates a protein nature of the effective compounds.

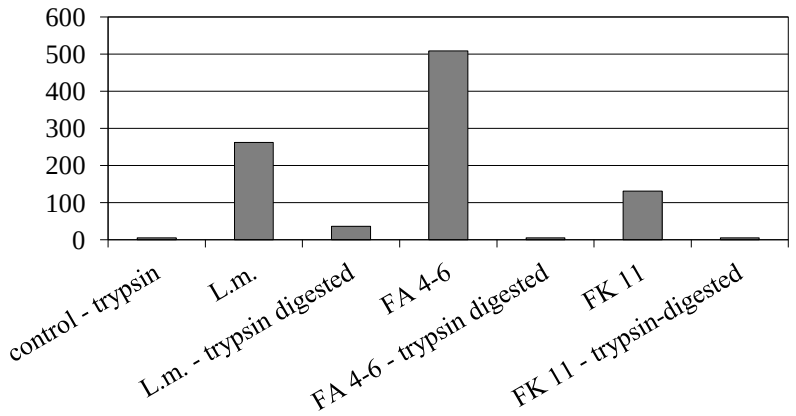
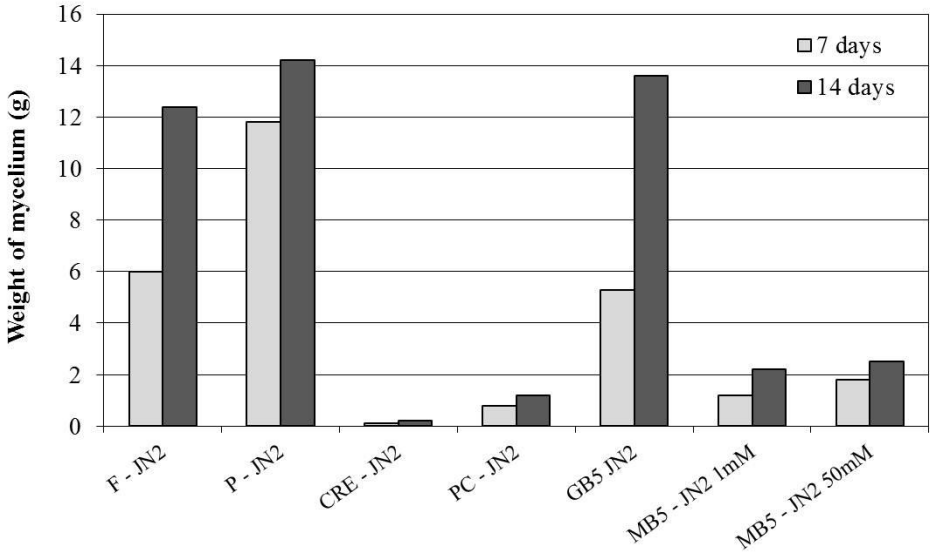


Figure 2. Expression of PR1 after elicitor treatment in *B.napus* cotyledons. L.m. – dialyzed cultivation medium (Fries), FA 4-6 – joined active fractions from anex columns, FK 11 – active fraction from catex columns, control-trypsin – trypsin water solution.

Cultivation media screening for proteinaceous elicitor production

Various cultivation media were tested for secreted *L. maculans* protein production. The goal was to find an alternative to the Fries (Férézou *et al.*, 1977) which is commonly used for *L. maculans* cultivation. This medium contains yeast extract, thus the presence of yeast proteins could produce misleading results. Five media not containing the yeast extract were chosen from those suitable for fungi cultivation. Gamborg B5 revealed to be comparable to Fries based on the biomass and protein production by *L. maculans* (Figure 3).

High biomass production in potato dextrose medium was not accompanied by protein production to the media, thus this medium was excluded from further experiments. Elicitors produced by *L. maculans* into Gamborg B5 medium after 14-days cultivation induced *PR1* expression in *B.napus* cotyledons (Figure 4).



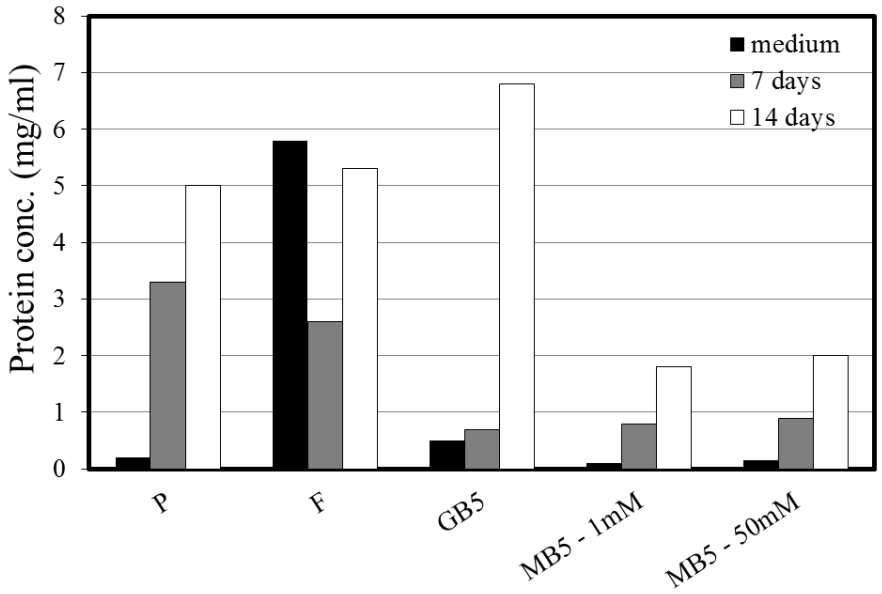


Figure 3. Fresh weight of mycelia (left) and protein content (right) in cultivation media: potato-dextrose (P), Fries (F), creatine sucrose (CRE), potato-carrot (PC), Gamborg B5 (GB5) and modified Gamborg B5 (MB5).

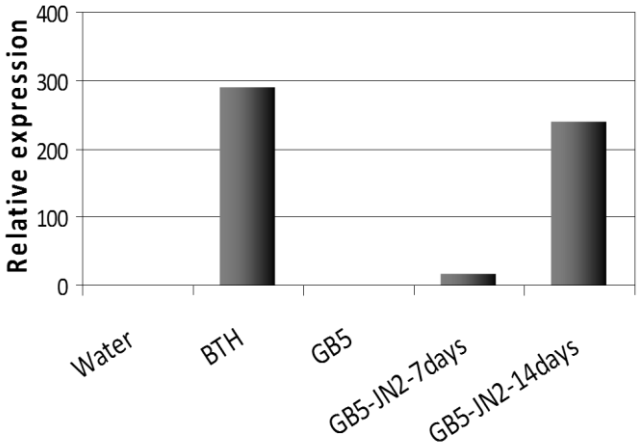


Figure 4. *PR1* expression induced by *L. maculans* cultivation medium Gamborg B5 (GB5) comparing to BTH.

Acknowledgements

The work was supported by a grant from the Ministry of Agriculture NAZV QH 81201, grant 522/08/1581 of the Czech Grant Agency, and MSM 6046137305 of Ministry of Education. Virulent (JN2) and avirulent (JN3) isolates of *L. maculans* kindly provided by Dr. Rouxel, INRA, Versailles, France.

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Sclerotinia stem rot

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Detection and quantification of airborne ascospores of *Sclerotinia sclerotiorum* by quantitative-PCR

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Abstract: A new SYBR-green quantitative PCR (qPCR) method was developed to quantify airborne inoculum of *Sclerotinia sclerotiorum*. This was tested on DNA extracted from spores deposited onto wax-coated plastic tapes, such as those used in Burkard (Hirst-type) spore traps and rotating-arm traps. A linear relationship between ascospore numbers and *S. sclerotiorum* DNA indicated a mean of 0.35 pg DNA per spore. The method could detect DNA representing as few as 2 ascospores. The technique was insensitive to DNA of the host plant, *Brassica napus*, and other plant pathogens, including *S. minor*, *S. trifoliorum*, *Botrytis cinerea*, *Leptosphaeria maculans* and *Pyrenopeziza brassicae*, and common airborne fungal genera such as *Cladosporium* and *Penicillium*. Specific quantification of *S. sclerotiorum* was achieved in the qPCR method by including a heating step to 79 °C to melt off any exogenous products such as primer dimers that would otherwise falsely contribute to the calculation of target DNA present. This step also eliminated products amplified from any DNA of *B. cinerea*, if present in the sample. The feasibility of using the method in disease forecasting schemes was tested using archived DNA from air samplers that operated at Rothamsted in April-May in three different seasons, which had contrasting *Sclerotinia* stem rot (SSR) epidemics; 2007 had a severe SSR epidemic in England and high numbers of airborne ascospores were trapped at Rothamsted; while both 2003 and 2004 had a very low incidence of SSR in England and low numbers of airborne ascospores trapped at Rothamsted. The severe SSR season of 2007 occurred throughout a large part of northern Europe and was not predicted in the UK by climate-based disease-forecasts. This study showed that there was no relationship between rainfall and numbers of airborne ascospores of *S. sclerotiorum* present at Rothamsted during the period of infection in the severe SSR season (2007). In addition to the example application tested in this study, the qPCR method reported here has potential to evaluate the presence and quantity of *S. sclerotiorum* in a wider range of environmental samples such as soil, seed or plant tissues such as petals or stems. In the case of airborne inoculum, further research is required to develop methods to rapidly apply the *S. sclerotiorum*-specific qPCR to air samples and to confirm that airborne inoculum is a reliable indicator of SSR risk by testing over a wider geographical range and number of growing seasons.

Key words: qPCR, air sampling, airborne spores, *Sclerotinia* stem rot

Introduction

Sclerotinia sclerotiorum causes *Sclerotinia* stem rot (SSR), a serious disease of oilseed rape (*Brassica napus*, canola). In western Europe, the severity of SSR epidemics varies with location and from year to year. Air-borne ascospores are released from fruiting bodies in the spring to initiate epidemics. Ascospores are not able to infect healthy plant tissues directly but do infect senescent tissues such as petals, which vector the pathogen onto lower leaves and branches to cause SSR. Petals provide an energy source required for infection of healthy tissues and since there is no known race-specific host resistance, control of SSR depends on the use of fungicides (Hegedus & Rimmer, 2007). Disease forecasting schemes have been developed to improve SSR control. These are mostly based on weather, which affects spore

release and crop growth stage (petal fall). However, variable responses to climatic factors in the pathogen population, along with diverse microclimates, can result in a wide variation in the timing of spore release. As a result, spore-based forecasting schemes may be more accurate in predicting epidemic risk.

The use of air sampling devices and their integration with different diagnostic methods has been reviewed recently (West *et al*., 2008). Ascospores of *S. sclerotiorum* are difficult to identify by microscopy as they are similar to other spores occurring in air. This article summarises the paper by Rogers *et al*. (2008), explaining the development of a new specific and quantitative PCR (qPCR) technique that can accurately quantify DNA of *S. sclerotiorum* in a background of DNA of other organisms (mainly plants and other fungi). A touch-down PCR assay for detecting spores of *S. sclerotiorum* that could be applied to air samples had already been reported by Freeman *et al*. (2002). However, that method provides only a qualitative indication of the presence or absence of inoculum, which has limited potential for forecasting and epidemiological studies compared to a quantitative diagnostic technique. This paper demonstrates that the new qPCR method can be applied to DNA extracted from air-sampled spores and discusses its potential for use in disease forecasting schemes.

Material and methods

Primer design and qPCR method

Two primers were selected, mtSSFor (5'-AGG TAA CAA GTC AGA AGA TGA TCG AAA GAG TT-3') and mtSSRev (5'-CCT TGT TTT TAG GGA CAG GCT TAA TGC-3') as having potentially good specificity towards *S. sclerotiorum* and no other sequences in the GenEMBL database (Rogers *et al*., 2009; 2011). The primers were first tested by PCR using the following conditions: 20 µl reaction volume containing 0.1 µmol of each primer, 10 µl 2 x PCR reaction buffer and RedTaq (1.5 mmol l^{-1} Mg^{2+}; Sigma, Gillingham, UK) and 1 µl DNA. PCR conditions were: 95 °C followed by 40 cycles of 94 °C for 1 min, annealing temperature 50 °C for 1 min and 72 °C for 1 min followed by a final extension step of 72 °C for 5 min and then the reaction was cooled to 4 °C.

For qPCR, Bioplastic (EU) 96 x 0.2 ml PCR plates capped with Bioplastic EU optical thin wall 8 cap strip low background (Bioplastics, Landgraaf, The Netherlands) were used with a qPCR machine Mx3000P (Stratagene, Amsterdam, The Netherlands) and SYBR green mix (Sigma-Aldrich, Gillingham, Dorset) was used for qPCR reactions. Concentrations of primers mtSSFor and mtSSRev were optimised to a concentration of 300 nmol each. Reference dye (ROX) was added according to the manufacturer's recommendations for use on the Mx3000P machine. The reaction mix was made up to 20 µl using ultra pure dH_2O. Quantiative PCR conditions comprised 95 °C for 2 min followed by 60 cycles of 95 °C for 15 s, 50 °C for 30 s and 72 °C for 30 s. A dissociation curve produced after the first reaction demonstrated that the qPCR product had a sharp peak at 81 °C. Therefore, an additional read step was added to the qPCR at 79 °C to melt off any exogenous products, such as primer dimers that may falsely contribute to the calculation of target DNA present. Reactions were performed in duplicate. The cycle threshold (CT) value for each qPCR was automatically calculated and analysed by the Stratagene MxPro version software (version 3.20; Stratagene).

DNA extraction

Two different methods were used to extract DNA from air samples, which were in the form of depositied spores on wax-coated plastic tape (7 x 48 mm), corresponding to a longitudinal half-section of a daily Burkard spore trap air sample (Figure 1). Each tape section was placed into a 2 ml screw-top tube. The first method had been used already to extract DNA from daily

outdoor Burkard trap samples from Rothamsted in 2003, 2004 and 2007 and was based on that of Williams *et al*. (2001) but with slight modifications (Rogers *et al*., 2008).

The second method was used subsequently and used 60 µl of a commercial detergent-based product, MicroLYSIS (Microzone, Haywards Heath, UK) combined with an additional step in which 0.1 g acid-washed, 400-600 µm diameter Ballotini Beads were added and shaken in a Fast Prep machine (FP120-Bio 101 – Qbiogene, California, USA) for 20 s at 4 ms^{-1}. The liquid was transferred to a 0.2 ml PCR tube and exposed to thermal cycling in a PCR block according to the manufacturer's protocol (65 ºC for 15 min, 96 ºC for 2 min, 65 ºC for 4 min, 96 ºC for 1 min, 65 ºC for 1 min, 96 ºC for 30 sec, 20 ºC hold). Additionally, 2 mg PVPP (polyvinylpolypyrrolidone, Sigma-Aldrich, Gillingham, Dorset) and 40 µl of TE buffer (10 mM, pH 8.0) were added, vortexed and spun at 13 K rpm for 15 min to remove polysaccharides, which can inhibit the PCR reaction. A 60 µl portion of the supernatant was removed to a new 0.2 ml tube to which 150 µl ethanol and 10 µl ammonium acetate (7.5 M, dissolved in water) was added, vortexed and spun at 13 K rpm for 15 min. The supernatant was discarded and the remaining pellet air dried and resuspended in 10 µl water. This was kept frozen at -20 ºC and 2.5 µl used per qPCR reaction.

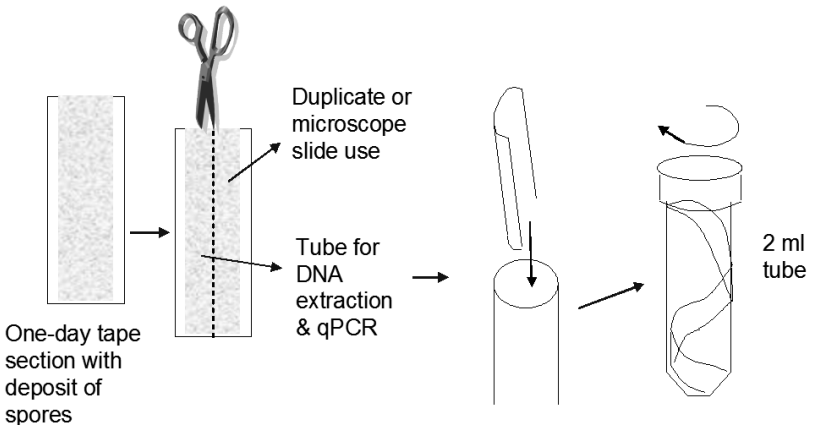

Figure 1. Stages in processing a Burkard spore trap air sample (waxed tape) for qPCR

Specificity and sensitivity testing

The specificity of the qPCR method was confirmed by testing DNA extracted from eight different UK isolates of *S. sclerotiorum*, and isolates of *S. minor*, *S. trifoliorum*, *Leptosphaeria maculans*, *Pyrenopeziza brassicae*, *Botrytis cinerea* and putative *B. cinerea* conidia from grapes, *Pelagonium*, and tomato, spores from two commonly airborne fungal genera (*Cladosporium* and *Penicillium*) and DNA from the host plant (*Brassica napus*).

Dynamics of DNA and crop disease

As ascospores of *S. sclerotiorum* are difficult to identify visually when mixed with other spores, DNA per ascospore was quantified from a regression of spore numbers against quantified DNA, with the spores deposited in laboratory conditions onto wax-coated plastic tapes as used in the Burkard spore sampler from a pure (mixed-isolate) *S. sclerotiorum* spore suspension. Half of different tape sections were counted by microscopy and the adjacent half-tape sections were processed for qPCR. Unexposed wax-coated tapes were used as negative controls. This was preferable to using outdoor air samples, with duplicate samples used for qPCR because confidence in spore counts when mixed with other spores was very low.

Outdoor air samples were taken from Burkard seven-day continuously recording spore samplers (Burkard Manufacturing Co. Ltd., Rickmansworth) operated at an area of grassland near to arable fields at Rothamsted in spring 2003, 2004 and 2007. The calculated amount of DNA per ascospore derived for lab conditions was applied to estimate spore numbers in each outdoor air sample. The incidence (% plants affected) of SSR in oilseed rape fields at Rothamsted (< 1 km from the air sampling site) was monitored each year and meteorological data were collected nearby at Rothamsted.

Results and discussion

Specificity and sensitivity of the qPCR method

The method quantified pure dilutions of *S. sclerotiorum* DNA over a range from 50 ng to 5 x 10^{-5} ng. There was a PCR product produced with *B. cinerea* but the heating step to 79 °C in the qPCR method was found to eliminate this from DNA quantification. The effect of *B. cinerea* DNA on the sensitivity of the qPCR method was tested, with no effect found except that due to 5 ng *B. cinerea* DNA (the highest amount added), which reduced the quantification of *S. sclerotiorum* at 5 x 10^{-5} ng (the lowest amount tested and close to the limit for detection of pure *S. sclerotiorum* DNA). No products were produced from DNA of any of the other species tested. Ascopore numbers estimated per tape sub-section by microscope were linearly related to *S. sclerotiorum* DNA quantities measured by qPCR in the ratio 0.35 pg DNA per spore (R^2 = 0.76, P < 0.001). The qPCR method could therefore detect as few as two spores in a background of other spores and plant pollens in air samples.

Weather, dynamics of ascospores and SSR incidence

S. sclerotiorum DNA quantities in air sampled during the oilseed rape flowering period were generally low in 2003 and 2004, which both had a very low incidence of SSR (< 0.1% plants affected). DNA amounts in 2003 corresponding to less than 4 ascospores per m^3 per day (and was usually zero) with even less present in 2004. In 2007, high levels of *S. sclerotiorum* DNA were found in air, despite a very dry April (Figure 2). That season had a relatively high incidence of SSR (over 5% of plants affected). Spores were released in early April, despite a lack of rainfall and this could be due to fruiting bodies being produced in soil that was still moist. However there was an unexpected peak of spores present in air in early May, when it had not rained much for more than four weeks.

General discussion

Quantitative PCR was found to be a rapid and accurate method to quantify inoculum of *S. sclerotiorum* and was more sensitive than the touch-down PCR method reported by Freeman *et al*. (2002). The method of Rogers *et al*. (2008) was able to detect DNA of *S. sclerotiorum* at amounts as low as 5 x 10^{-4} ng (0.5 pg; or representing 1.4 ascospores) in the presence of DNA of *B. cinerea*. The qPCR method used on artificially inoculated plastic-tape sections produced under lab conditions, found 0.35 pg DNA per ascospore of *S. sclerotiorum*. The variability in the relationship shown (R^2 = 0.76) may be due to an uneven distribution of the sprayed ascospores onto the tape surface leading to slight differences in numbers of ascospores between those counted by microscopy on one half of the tape and those detected by qPCR on the other half. The results of tests of outdoor air samples show that this method has potential for use in inoculum-based forecasting schemes. However, data from more sites and seasons are required to demonstrate conclusively the relationship between airborne inoculum and this disease. The new qPCR method has already demonstrated the presence of

airborne inoculum when conditions were very dry (in 2007). This may mean that inoculum can be blown from distant sources. As the epidemic was widespread over north-western Europe in 2007, it may mean that relatively few air samplers would be needed to indicate inoculum presence at regional scales. To provide an effective warning of inoculum presence, further work is required to develop methods to process samples and disseminate results quickly. The qPCR technique could also be used to quantify *Sclerotinia sclerotiorum* in other types of environmental sample such as seed or soil or to assess growth of the pathogen in OSR varieties as a method to assess host resistance.

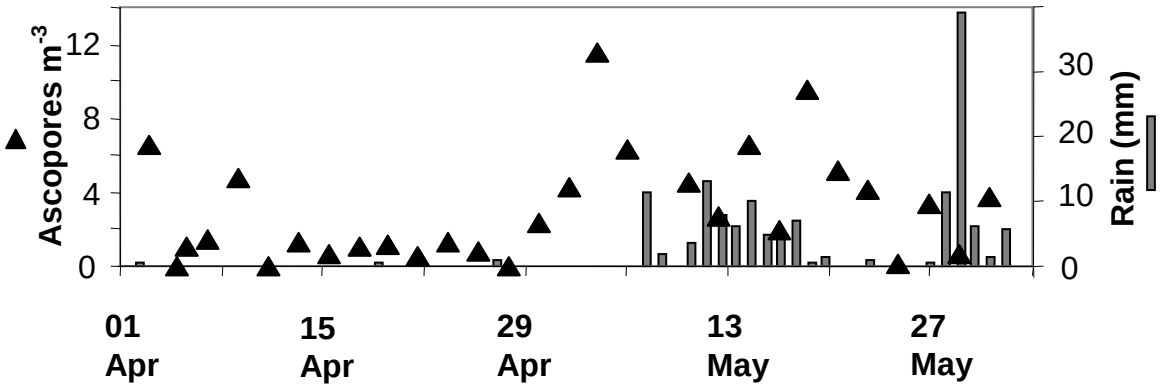

Figure 2. Changes in amount of rainfall and airborne concentrations of *Sclerotinia sclerotiorum* ascospores estimated from their DNA at Rothamsted in 2007.

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Economic gains and Integrated Pest Management: An application to the *Sclerotinia* and the Canola Crop

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Abstract: Agronomic experimental data are used for simulating some economic implications linked to an integrated pest management applied to *Sclerotinia* stem rot in France. This integrated pest management is a diagnostic test allowing farmers to reduce their fungicide application for canola. Profit estimations for farmers are used for determining whether or not farmers would adopt this integrated pest management. Before 2007, simulations show that the use of a *Sclerotinia* diagnostic test provides economic gains for farmers slightly lower than the common used practice of preventive spraying of fungicides. However, the canola price increased in 2007 makes the diagnostic test less attractive for farmers compared to the systematic fungicide application. Various political scenarios including a test subsidy or an insurance program linked to the diagnostic test are examined. We show that both instruments would lead to the use of the diagnostic test, even if, for the regulator, the test subsidy is less costly than the insurance program linked to the diagnostic test.

Key words: *Sclerotinia*, integrated pest management

Introduction

Sclerotinia sclerotiorum (*Sclerotinia*, hereafter) is a fungal disease affecting canola (oilseed rape) crops and negatively impacting yields. This fungus generates black bodies called sclerotia that can remain in the soil for several years. Under humid weather conditions at flowering, sclerotia produce spores that infect the crop and reduce yields (Taverne and Penaud, 2001). In order to cope with this risk, farmers mainly rely on preventive fungicides use at flowering, since no curative protection exists once the fungus infects. The preventive nature of this protection implies potential fungicide waste and adverse effects on non-target organisms.

For many farmers, fungicides against *Sclerotinia* are essential to provide high crop yields with canola. However, fungicides can lead to dangerous side effects such as toxicity for the applicator, contamination of the water cycle, and toxicity for honey bees. Excess fungicide application may also encourage the development of pesticide resistance, jeopardizing future crop protection as was the case for *Sclerotinia* with respect to carbendazime in the nineties and recently with the imides. This explains why regulators or/and producers try to turn to alternative programs for limiting fungicides use.

Integrated pest management substitutes information about the state of the crop and/or crop rotation for chemical pesticides. For *Sclerotinia*, control crop rotation seems unrealistic because of the persistence of sclerotia in the soil for relatively long periods. An experimental tool for providing accurate information before spraying fungicides was developed in France over the past few years. A diagnostic test about the state of *Sclerotinia* before spraying

fungicide was developed by CETIOM since 2001 (CETIOM, 2006). This diagnostic test was a promising alternative to the systematic use of fungicide. This issue will gain momentum since the European Commission wants to enforce ambitious pest management programs for reducing chemical uses by 2014 (EC, 2006). Today almost all French farmers systematically use chemical fungicides for eliminating *Sclerotinia* on canola (Charbonnaud, 2007).

The diagnostic test is based on petal plating on agar medium providing imperfect information to farmers before spraying the fungicides. The measured proportion of infected flowers given by the diagnostic test allows farmers to streamline the fungicide use. However, diagnostic errors occur when the diagnostic test predicts (i) a high risk of disease while the true disease incidence is low before harvest, or when the test predicts (ii) a low risk of disease while the true disease incidence is high before the harvest. Errors mainly depend on climatic factors, in particular rainy conditions that influence the disease development after the diagnostic test. Farmers face risks in their decisions to comply with the diagnostic test advising the absence of fungicides application. One solution for mitigating the risk consists of offering an insurance program to cover subsequent yield loss, in cases where the test predicts a low risk of disease leading to the absence of application, while the true disease incidence is high before harvest. The diagnostic test is still experimental, but available data may be useful for forecasting eco-conditional policies. The purpose of this paper is to evaluate the impact of a diagnostic test on farmers' decisions and/or the related policy. Different options regarding the regulatory policy are also estimated.

The results of this paper are as follows. A diagnostic test could contribute to improve the management of crop diseases even if the systematic application of fungicide economically dominates the use of the diagnostic test that can streamline the fungicide use. Various political scenarios including insurance linked to the diagnostic test or a test subsidy are simulated. We show that both instruments would lead to the use of the diagnostic test, even if, for the regulator, the test subsidy is less costly than the insurance program.

Despite various limitations coming from data and methodology, these simulations provide credible suggestions for the choice of instruments by a regulator aiming at implementing eco-conditionality. The results may help the decision maker streamline the fungicide spray.

This paper contributes to various aspects of regulatory debates. From a methodological point of view, our paper adds to the literature by directly taking into account agronomic experimental results of a specific protection problem that could pave the way for eco-conditional policies. This methodology supports public debates about the best way to promote clean crops. Despite limitations, different regulatory scenarios may be tested *ex ante*, namely before a policy implementation. We add to the literature by considering regulatory instruments such as insurance often overlooked by previous contributions (see for instance Fernandez-Cornejo and Smith, 1998, and Lichtenberg and Velderman Berlind, 2005).

Material and methods

Data

Data used in this study were obtained from canola experiments carried out by CETIOM over 7 years (2001-2007) on farms in France. Each experiment consisted of around 20 meters of unsprayed field that gave information about the disease in the absence of fungicide application. The first column of Table 1 indicates the number of unsprayed fields (or observations) that were available each year.

The diagnostic test based on flowers plating on agar medium provides imperfect information to farmers about the disease before their decision about the fungicide application. All unsprayed fields were tested at the period preceding the spray decision for other fields. The proportion of infected flowers measured by the test before fungicide application can be used to make an assessment of the need for crop protection in agricultural fields. The measured proportion by the diagnostic test is compared with an optimal threshold for knowing when it is necessary to apply fungicides.

The optimal threshold was determined by the CETIOM at 30% (CETIOM, 2006). If the proportion of infected flowers given by the diagnostic test is lower than 30%, the optimal rule consists in avoiding application. Conversely, farmers are advised to apply fungicides if the proportion of infected flowers given by the diagnostic test is greater than 30% (Makowski *et al.*, 2008). The second column of Table 1 shows this proportion of infected flowers greater than 30%. Note that the use of fungicides, as triazole or carboxamide, also entails a "greening effect", namely an increase of the yield equal to one quintal and a half per hectare (CETIOM, 2007). In other words, the absence of fungicide application misses this effect.

Table 1. Number of observations

	Number of observations	Test diagnostic (> 30%) fungicide application	High disease incidence (> 10%)
2001	159	23%	11%
2002	44	50%	32%
2003	62	18%	10%
2004	84	85%	33%
2005	247	82%	19%
2006	196	74%	49%
2007	150	72%	69%

Sources: CETIOM

The effective disease incidence was observed by CETIOM at the end of the growing season, about 3 weeks before harvest. Numerous plants were selected randomly in each untreated plot, and were rated for the presence of disease. Disease incidence for each field was calculated as the percentage of plants with symptoms of *Sclerotinia* stem rot.

The fields are divided in two categories according to their disease incidence level. For a disease incidence lower than 10%, farmers incur no yield loss. The 10% threshold represents an injury level above which a fungicide would be needed to control the disease. Between 10% and 19% of disease incidence, a loss of one quintal per hectare is incurred. An additional loss of one quintal per hectare is added to the previous loss for every 10% increments regarding the disease incidence (CETIOM, 2006). It means that for a disease incidence between 20% and 29% (respectively, x.10% and [(x+1).10-1]%), the yield loss is equal to 2 (respectively x) quintals per hectare. The third column of Table 1 presents the percentage of fields with a high disease incidence, namely a disease incidence greater than 10%.

The third column of Table 1 shows a large inter-year variability regarding the high disease incidence, since the weather conditions under the absence of fungicides applications are crucial. Note that for 5 consecutive years more than 65% of the unsprayed fields do not need any fungicide application because of a low-incidence of disease (lower than 10%). This

is an important detail when we know that almost all French farmers systematically use chemical fungicides for controlling *Sclerotinia* (Charbonnaud, 2007). In other words, many fungicide applications are unrequired *ex post*, even if preventive action via the fungicide application is *ex ante* profitable for farmers.

From Table 1, it is clear that many cases with farmers spraying preventively are unrequired, which shows that private or regulatory actions are possible, in particular via the diagnostic test. Complying with the decision rule linked to the test (namely, avoiding fungicide application if the test is lower than 30%) is risky. Diagnostic errors occur when a diagnostic test predicts a high risk of disease when the true disease incidence is low in the considered field, or when the test predicts a low risk of disease when the true disease incidence is high. Errors mainly depend on climatic factors, in particular rainy conditions that influence the disease development after the diagnostic test. Farmers face risks in their decisions to follow up the diagnostic test and the associated decision rule.

Method for estimating the economic gains for farmers

We use a very simple model for computing the net-pecuniary gains for farmers who are risk neutral (this simplifying assumption is discussed at the end of the paper). We focus on the pecuniary gains, namely the benefits for which values are determined by market prices and thus can be denominated in Euro in a straightforward manner. We now turn to the definitions of the net gains where the absence of application, the systematic application and the use of the diagnostic test before spraying are compared. For simplicity, we focus on the per-hectare gains of a representative farmer by abstracting from farmers' heterogeneity linked to the farms size, the technical knowledge, the risk aversion.

The loss per hectare linked to the complete absence of application for a given year is given by $\ell_0 = -p_c\left(\sum_{i=1}^{10}\lambda_i.i + G\right)$, where p_c is the canola price at the farm gate for a given year. The parameter *G* measures the lack of "greening effect" when no fungicide is used. λ_i is the probability of losing *i* quintals per hectare. This probability of losing *i* quintals per hectare is directly calculated from the effective disease incidence given by the CETIOM database. This probability λ_i is equal to the percentage of plants with a disease incidence level between i.10% and (i.10+9)% among all the plants observation for a given year. Recall that the third column of Table 1 presented fields with high disease incidence, namely a disease incidence greater than 10%, which is equal to $\sum_{i=1}^{10}\lambda_i$ with our notations.

The systematic application of fungicides allows famers to reduce the quintal losses. The cost of fungicide application and the residual loss per hectare linked to the systematic application for a given year is given by $\ell_1 = -(1-E_f).p_c\left(\sum_{i=1}^{10}\lambda_i.i\right) - F - A$, where E_f is the percentage of efficiency coming from the fungicide, *F* is the fungicide cost and *A* is the cost of the fungicide application (namely the material and application costs). With a percentage $(1-E_f)$, the application is useless and losses occur. Other parameters were detailed in the previous paragraph.

For a given year and for a farmer, the net-pecuniary gain per hectare coming from a systematic application compared to the absence of application is

$$\Delta\pi_1 = \ell_1 - \ell_0 = E_f.p_c\left(\sum_{i=1}^{10}\lambda_i.i\right) - F - A + G.p_c\,. \qquad (1)$$

This value is equal to the loss reduction linked to the systematic fungicide application. A positive value of $\Delta\pi_1$ means that the fungicide application is profitable, since the loss ℓ_1 linked to the systematic treatment including the fungicide cost is lower than the loss ℓ_0 linked to the absence of application.

We now turn to the loss linked to the diagnostic test and the related decision rule, namely no application if proportion of infected flowers given by the test is lower than 30%. For a given year, the loss/cost of using the test is given by the value $\ell_2 = -(1-\gamma).p_c\left(\sum_{i=1}^{10}\underline{\lambda_i}.i + G\right) - \gamma.\left[(1-E_f).p_c\left(\sum_{i=1}^{10}\overline{\lambda_i}.i\right) + F + A\right] - T$, where T is the cost of the test, γ is the proportion of infected flowers given by the diagnostic test with a proportion greater than 30%, for which it is useful to apply fungicides with values of E_f, F and A detailed above. For each year, the value of γ are detailed in the second column of Table 1. It is assumed that the probability of having infested flowers is directly equal to this proportion γ. The probabilities $\overline{\lambda_i}$ of loss per hectare are conditional to the probability γ of having a diagnostic test greater than 30% and are also computed with the CETIOM data.

The value $(1-\gamma)$ is the proportion (and the probability) of infected flowers given by the diagnostic test with a proportion lower than 30%, leading to the absence of treatment. The probabilities $\underline{\lambda_i}$ of loss per hectare are conditional to this probability of having a diagnostic test lower than 30%. Data show that $\underline{\lambda_0} > \lambda_0 > \overline{\lambda_0}$ for a low disease incidence implying no yield loss, namely a disease incidence lower than 10%. Data also show that $\underline{\lambda_i} < \lambda_i < \overline{\lambda_i}$ with $i \in \{1,10\}$ for a high disease incidence implying yield losses, namely a disease incidence greater than 10%. Eventually, using the value λ_i under the complete absence of application (see above), the following equality $\lambda_i = (1-\gamma)\underline{\lambda_i} + \gamma.\overline{\lambda_i}$ is satisfied.

For a farmer, the net pecuniary gain per hectare coming from the diagnostic test compared to the absence of application is

$$\begin{aligned}\Delta\pi_2 = \ell_2 - \ell_0 = &-(1-\gamma).p_c\left(\sum_{i=1}^{10}\underline{\lambda_i}.i + G\right)\\ &-\gamma.\left[(1-E_f).p_c\left(\sum_{i=1}^{10}\overline{\lambda_i}.i\right) + F + A\right] - T + p_c\left(\sum_{i=1}^{10}\lambda_i.i + G\right).\end{aligned} \qquad (2)$$

A positive value of $\Delta\pi_2$ means that the diagnostic test and the conditional fungicide application are profitable, since the loss ℓ_2 including the fungicide cost and the cost of the diagnostic test is lower than the loss ℓ_0 coming from the absence of application.

For a farmer, the net pecuniary gain per hectare coming from a systematic fungicide application compared to the diagnostic test and the advised fungicide application is

$$\Delta\pi_3 = \ell_1 - \ell_2 = -(1-E_f).p_c\left(\sum_{i=1}^{10}\lambda_i.i\right) - F - A + (1-\gamma).p_c\left(\sum_{i=1}^{10}\underline{\lambda_i}.i + G\right) + \gamma.\left[(1-E_f).p_c\left(\sum_{i=1}^{10}\overline{\lambda_i}.i\right) + F + A\right] + T \quad . \quad (3)$$

A positive value of $\Delta\pi_3$ means that the systematic application is profitable compared the diagnostic test, since the loss the loss ℓ_1 linked to the systematic treatment is lower than to the loss linked to the use of the diagnostic test and the conditional fungicide treatment for a proportion of infected flowers greater than 30%.

The values of the parameters used for the simulations are given in Table 2 where two fungicides are taken into account. In our simulations, the canola price p_c and the probabilities $\lambda_i, \underline{\lambda_i}, \overline{\lambda_i}, (1-\gamma), \gamma$ are specific to each year over the period to 2001-2007. Conversely, the values of other variables *G* to *F* in Table 1 are constant over the period 2001-2007.

Table 2. Values of variables

Variable	**Description**	**Values**
p_c	Canola prices 2001-2007 (farm gate) mean over the period	23€
G	Green effect (quintal per hect.)	1.5
A	Cost of application (per hect.)	8€
T	Cost of the diagnostic test (per hect.)	5€
Fungicide with triazole		
E_f	Application efficiency of the fungicide	0.65
F	Cost of fungicide (per hect.)	30€
Fungicide with carboxamide (PictorPro)		
E_f	Application efficiency of the fungicide	0.85
F	Cost of fungicide (per hect.)	40€

Sources: Rica (2007), CETIOM (2007), Duroueix (2007).

Note that, for the net gains given by our estimations, we abstracted from other crop disease such as mildew and *Alternaria* for which both fungicides considered in Table 2 are useful and have a positive impact on crop yield. We did not consider these diseases because of a lack of precise data. The net gains of the fungicides applications that we computed are slightly underestimated since these other diseases are not taken into account.

Results and discussion

Estimations of $\Delta\pi_1$, $\Delta\pi_2$ and $\Delta\pi_3$ are presented in Table 3 for each year and for the application of two different fungicides. Recall that we abstracted from risk aversion by farmers. Clearly, from Table 3, the net-pecuniary gain per hectare for farmers depends on the year because of different disease incidences across the years.

Table 3. Net pecuniary gains per hectare (in €)

	Systematic application versus nothing $\Delta\pi_1$	Diagnostic test versus nothing $\Delta\pi_2$	Systematic application versus diagnostic test $\Delta\pi_3$
Fungicide with Triazole			
2001	–4.20	–5.69	1.49
2002	5.48	2.31	3.16
2003	–2.76	–3.80	1.03
2004	0.10	–3.80	3.91
2005	–4.60	–7.60	3.00
2006	9.25	5.27	3.97
2007	56.78	45.99	10.78
Fungicide with Carboxamide			
2001	–13.59	–7.74	–5.84
2002	–1.01	0.61	–1.63
2003	–12.12	–4.95	–7.17
2004	–7.76	–10.21	2.49
2005	–13.18	–14.93	1.75
2006	4.91	3.16	1.75
2007	63.01	53.22	9.70

From the first two columns of Table 3, it is clear that the benefit of a systematic fungicide application or a diagnostic test versus the absence of fungicide application depends on the importance of the *Sclerotinia* disease. If we focus on the triazole application (namely, the "cheap" fungicide at the top of Table 3), it was optimal to avoid fungicide application for the years 2001, 2003 and 2005, since the incidence of the disease was relatively low these years (see the third column of Table 1 where the high disease incidence was low). For the other years, it was optimal to systematically apply triazole fungicides. These results explain the systematic fungicide application currently observed in France, since the introduction of a slight risk aversion by farmers and/or the consideration of other disease such as mildew and *Alternaria* will lead farmers to forget performances from 2001, 2003 and 2005, for focusing on years where the treatment was useful. In other words the slight benefits linked to the absence of application in 2001, 2003 and 2005 under risk neutrality vanish when risk aversion is introduced.

The carboxamide application (namely the expensive fungicide at the bottom of Table 3) seems less attractive than the triazole despite a better efficiency for controlling *Sclerotinia*. The exceptions are for the years 2006 and 2007 for which the *Sclerotinia* incidence was very intense. Net gains for farmers were relatively large in 2007 for both triazole and carboxamide applications, since the canola price increases 40% compared to 2006, partially because of biodiesel demand in France. Clearly, larger commodity prices imply a larger use of chemical products for maintaining yields and boosting farmers' profits.

Eventually, the diagnostic test and the rule linked to this test (namely, avoiding fungicide application if the flower test is lower than 30%) almost never emerge as the best solution for

farmers except for the year 2002 with the use of carboxamide only, since the second column is positive with $\Delta\pi_2 = 0.61$ and the third column is negative with $\Delta\pi_3 = -1.63$. For other years, the diagnostic test is dominated by either the absence of application or the systematic application.

The third column of Table 3 shows that the systematic application often dominates the use of the diagnostic test. Without additional incentives for farmers to use the diagnostic test, they would prefer the systematic fungicide application. The use of this test that offers both advantages for limiting resistances and preserving the environment requires regulatory intervention.

Possible regulatory choices

We now focus on regulatory choices that could lead some farmers to use the diagnostic test and comply with the optimal rule linked to this test (namely, avoiding fungicide application if the test is lower than 30%). We focus on two instruments directly linked to this test, namely (1) a subsidy of the diagnostic test and (2) a public insurance in case of a high disease incidence (greater than 10%) with a diagnostic test (lower than 30%) advising to avoid fungicide application. We assumed that the cost linked to the greening effect $G.p_c$ is also covered by this insurance. This public insurance fully covers the loss linked to the absence of application coming from a test lower than 30%. For simplicity, we abstract from a partial coverage of the loss. For both case, we assumed an administrative cost equal to 10€ per hectares, because of the servicing and the monitoring guaranteeing the absence of application when the test is lower than 30%.[1]

For both instruments, we may rewrite the loss/cost ℓ_2 of using the diagnostic test (see the previous section for a detailed definition). With a test subsidy, the cost T is not incurred by the farmer since the regulator finance it. Assume that $I_T = 1$ if the test is financed by the regulator and $I_T = 0$ otherwise. With the public insurance, the yield loss is fully covered by a public fund in case of a high disease incidence (greater than 10%) with a diagnostic test (lower than 30%) advising to avoid fungicide application. Assume that $I_i = 1$ if the insurance is decided by the regulator and $I_i = 0$ otherwise. Under regulation, the loss/cost ℓ_2 of using the diagnostic test is

$$\widetilde{\ell_2} = -(1-I_i)(1-\gamma).p_c\left(\sum_{i=1}^{10}\underline{\lambda_i}.i+G\right)-\gamma.\left[(1-E_f).p_c\left(\sum_{i=1}^{10}\overline{\lambda_i}.i\right)+F+A\right]-(1-I_T)T \qquad (4)$$

Under regulation, the net pecuniary gain coming from a systematic fungicide application compared to the diagnostic test and the advised fungicide application (for a test greater than 30%) is

$$\begin{aligned}\Delta\widetilde{\pi_3} = \ell_1 - \widetilde{\ell_2} = &-(1-E_f).p_c\left(\sum_{i=1}^{10}\lambda_i.i\right)-F-A+(1-I_i)(1-\gamma).p_c\left(\sum_{i=1}^{10}\underline{\lambda_i}.i+G\right)\\ &+\gamma.\left[(1-E_f).p_c\left(\sum_{i=1}^{10}\overline{\lambda_i}.i\right)+F+A\right]+(1-I_T)T.\end{aligned} \qquad (5)$$

A positive value means that the systematic application is profitable compared the diagnostic test, since the loss ℓ_1 linked to the systematic fungicide application is lower than

[1] We abstract from private insurances that are unlikely to be offered by private companies. As Babcock (2007 p. 5) notes "experience has shown that insurance benefit alone is insufficient motivation for most farmers to buy crop insurance."

loss ℓ_2 linked to the use of the diagnostic test. The diagnostic test is selected by farmers for a negative value of $\Delta\pi_3$.

Table 4 focuses on the impact of the insurance program ($I_i = 1$ and $I_T = 0$), while Table 5 focuses on the impact of the test subsidy ($I_i = 0$ and $I_T = 1$). Estimations of $\Delta\pi_3$ are presented in Tables 4 and 5 for each year and for the application of two different fungicides. The second column shows the per-hectare transfer offered to farmers. The third column presents the overall cost of the policy (including the administrative cost of 10 € per hectares), when we consider all French farm producing canola. We considered 65 478 farms with an average of 16 hectares and producing canola in France for determining this overall cost (Desbois and Legris, 2007).

Table 4. Impact of a public insurance linked to the diagnostic test (in €)

	Systematic application versus diagnostic test $\Delta\pi_3$	Expected indemnity payment per hectare	Overall cost in France
Fungicide with Triazole			
2001	–24.89	26.38	38 120 829
2002	–14.34	17.50	28 819 844
2003	–26.14	27.18	38 954 695
2004	–0.76	4.67	15 377 115
2005	–2.80	5.80	16 557 351
2006	–5.53	9.51	20 443 698
2007	–8.81	19.60	31 010 380
Fungicide with Carboxamide			
2001	–32.23	26.38	38 120 829
2002	–19,14	17.50	28 819 844
2003	–34.35	27.18	38 954 695
2004	–2,22	4.67	15 377 115
2005	–4,05	5.80	16 557 351
2006	–7,76	9.51	20 443 698
2007	–9,80	19.60	31 010 380

Both tables show that both instruments would lead farmers to use the diagnostic test for improving the fungicide management (since the first column of each table is negative except for 2007 in Table 5). Results in terms of farmers' incentives are relatively close for both instruments based on the comparisons of the first columns of Table 4 and 5. Farmers would prefer the insurance program of Table 4 leading to higher profits. The subsidy detailed in Table 5 is less costly in terms of monetary transfers compared to the insurance program detailed in Table 4. For the taxpayer, the insurance program is particularly costly for the years 2006 and 2007 for which the Sclerotinia incidence was very high.

Table 5. Impact of a diagnostic test subsidy (in €)

	Systematic application versus diagnostic test $\Delta\pi_3$	Diagnostic test subsidy per hectare	Overall cost of in France
Fungicide with Triazole			
2001	–3.50	5	15 714 720
2002	–1.83	5	15 714 720
2003	–3.96	5	15 714 720
2004	–1.08	5	15 714 720
2005	–1.99	5	15 714 720
2006	–1.02	5	15 714 720
2007	5.78	5	15 714 720
Fungicide with Carboxamide			
2001	–10.84	5	15 714 720
2002	–6.63	5	15 714 720
2003	–12.17	5	15 714 720
2004	–2.55	5	15 714 720
2005	–3.24	5	15 714 720
2006	–3.24	5	15 714 720
2007	4.79	5	15 714 720

Tables 4 and 5 show that public monetary transfers help farmers to adopt the diagnostic test. The model used for the simulations was admittedly simple and extensions should be considered. First, the persistence of sclerotia in soil over long periods also favours the systematic application of fungicide by farmers. If a farmer already faced up a *Sclerotinia* problem, he will systematically spray fungicide the following year because of the persistence.

One limit of this study also comes from the absence of the non-pecuniary gains for famers in the net gains detailed by equations (1) to (5). A pecuniary-net gain is a rough measure that abstracts of many other costs/benefits without a direct monetary measure. Non-pecuniary benefits are those benefits perceived by farmers but not traded in markets and thus there is not an appropriate market price that can be used to value the changes in these benefits. As a result, they are much more difficult to value. Contingent valuation or experimental methods may be employed to obtain an estimate of the value farmers place on them. These non-pecuniary benefits could include increased human safety and environmental improvements from lower fungicide use, risk aversion and the value of convenience. Components of the convenience value could include a simpler production system, less concern about the timing of applications and time savings. Some of the components of convenience may be able to be priced from market transactions, but most cannot.

Risk aversion also matters for explaining farmers decisions. Risk aversion is highly heterogeneous among farmers (Bar-Shira *et al.*, 1997; Chavas and Holt, 1996). Risk aversion favours the use of fungicide as an “insurance” mechanism. Our assumption of risk neutrality makes the farmers’ reluctance to use the flowers test more conservative: if buyers are risk averse, the desire for and the benefits from systematic application of a fungicide increase.

Under risk aversion, results of Table 3 are reinforced with risk aversion and public programs in Tables 4 and 5 for giving an incentive to famers are more costly. The non-pecuniary gains and the risk aversion should be taken into account in future studies.

Conclusions

This paper improves our understanding of how some instruments influence farmers' behaviours. We showed that a diagnostic test could contribute to improve the management of crop diseases even if the systematic application of fungicide economically slightly dominates the use of the diagnostic test that can streamline the fungicide use. On a voluntary basis, an integrated pest management selected by farmers would not emerge.[2] Various political scenarios including insurance linked to the diagnostic test or a test subsidy were simulated. We showed that both instruments would lead to the use of the diagnostic test, even if, for the regulator, the test subsidy is less costly than the insurance program.

The main implication of this paper is the need for public subsidization for providing incentives to famers to adopt the integrated pest management allowing them to streamline their use of fungicides. A complete cost-benefit analysis should take into account the results of this paper for determining the optimal regulation to promote methods respecting the environment and limiting the use of chemical pesticides.

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[2] One extension would consist in considering the value given by farmers to the problem of pesticide resistances, which could lead some farmers to streamline their chemical use via the voluntary selection of an integrated pest management.

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Use of infection criteria from SkleroPro to identify infection events for *Sclerotinia* stem rot in England, 1991-2007

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Abstract: Stem rot (*Sclerotinia sclerotiorum*) has increased in importance in England recently, but risk assessment and decisions on fungicide use remain difficult for individual crops. Stem rot levels in winter oilseed rape in 2007 were the highest yet recorded and the most severe since 1991. This prompted an investigation of the factors affecting the development of the 2007 epidemic. Petal tests indicated that inoculum was present despite very low rainfall in the spring. Hourly weather data for the flowering periods in 2006 (a 'non-epidemic' year) and 2007 were collated from a range of metereological stations to represent the main areas of production. The number of periods during flowering that met the infection criteria used in SkleroPro (minimum of 23 h with relative humidity > 80% and temperature > 7 °C) were identified. Further comparisons of infection events were made between ADAS Rosemaund, Hereford (west) and ADAS Boxworth, Cambridge (east) for the years 1991-2007. The SkleroPro infection criteria were useful for identification of infection events and interpretation of fungicide experiments where sclerotinia inoculum was present. At ADAS Boxworth where little sclerotina developed in most years, inoculum was considered to be limiting. The development of more effective guidance for stem rot management will require quantification of inoculum and prediction of infection events.

Key words: *Sclerotinia sclerotiorum*, infection period, disease risk, weather

Introduction

Stem rot caused by *Sclerotinia sclerotiorum* is a common disease of oilseed rape in the UK, though there have been only limited numbers of severe attacks in England in most years (Hardwick and Turner, 1995). There were numerous severe attacks in winter oilseed rape in Scotland in 2006 and in southern and western England in 2007 (Gladders *et al*., 2008) and more widespread problems in England in 2008. Defra-funded oilseed rape disease surveys reported by CropMonitor (www.cropmonitor.co.uk), indicated that the stem rot epidemics in 2007 and 2008 were the most serious since 1991. *Sclerotinia* stem rot has now become an economically important disease for many growers and improved guidance on its management is required.

Some analyses of the 1991 epidemic have been reported (Gladders *et al.*, 1993). However, there was more monitoring and disease data for 2007 than 1991 and this provided the first opportunity to analyse a severe epidemic in England since 1991. A new sclerotinia infection model known as SkleroPro has been developed in Germany (Koch *et al.*, 2006). Controlled environment studies showed that stem infection by ascospores required a minimum period of 23 hours with relative humidity above 80% at 7 °C. Under conditions in Germany, the incidence of sclerotinia at harvest was significantly correlated with number of 'infection' hours above 23 during flowering. This paper reports on the occurrence of infection periods as defined by SkleroPro at sites in England and examines their use to explain stem rot epidemics.

Material and methods

Weather and crop data

Fifteen weather sites were chosen in England that provided a geographical spread similar to areas in which oilseed rape was grown (Table 1). Hourly weather data for April, May and June in 2006 and 2007; rain, relative humidity and maximum and minimum temperatures were collected and assembled in a Microsoft Excel database. Where small numbers of missing datapoints were present, averages of the nearest data points were used to estimate the missing data records. A visual basic macro was written to extract information on the occurrence and duration of weather conditions favourable for sclerotinia development based on the criteria used in the German model SkleroPro (Koch *et al.*, 2006). The macro calculated when temperature was above 7 °C and relative humidity was > 80% for each site and the number of consecutive hours with these conditions summed. In the model, sclerotinia infection periods require a minimum of 23 consecutive hours at >80% r.h. when temperatures are above 7 °C. Further analyses were then limited to events occurring during the main flowering period, defined from ADAS crop and experimental reports (Table 2).

Long term analyses

Hourly weather data available for ADAS Boxworth, Cambridge (east) and ADAS Rosemaund, Hereford (west) was used for most of the long-term analyses. The Boxworth dataset was enhanced with data from Wyton for 1990 to 1993 and Church Lawford weather was used for Rosemaund from 1991 to 1998. Missing data for rainfall for Rosemaund in 2007 was obtained from Credhill. Short periods of missing data in April 2003, 2004 and 2005 were supplemented by data from the Cambridge University Weather station. Weather records have been analysed to identify the number of infection events (using the SkleroPro model criteria) and their duration during April to June each year from 1991 to 2007 and related to stem rot incidence recorded in untreated crops on these ADAS farms each year during 1991-2007. Data for petal tests for sclerotinia in some of these crops was also available (Davies *et al*., 1999).

Results and discussion

In 2006, flowering was later than usual and continued mainly during May. There were infection ‘events’ on 6 or 7 May at seven locations followed by further events at mid-May at 13 locations. Boxworth, Cambs had seven infection ‘events’, only Bracknell had none at all (Table 1). In 2007, flowering continued from early April until mid-May. Conditions were suitable infection in late April (mainly 22 or 23 April) at eight sites distributed across the east, south-east and south-west regions (Table 1). These were followed by one or two infection events during 8-16 May, though sites had these on different dates. Brize Norton and Coltishall had no infection events in 2007 whilst Bracknell, Herstmonceaux and Newcastle had no events during May (Table 1).

Early sclerotinia infection is usually the most important as plants are likely to ripen prematurely and produce little yield. There was potential for early infection in 2007 during 22-23 April at some locations (Table 1) and stem symptoms were confirmed in the Hereford area in early May. More widespread infection appears to have occurred in mid-May given the observations that there was significant new development of stem rot after the end of May (Ritchie *et al*., this vol). Some sites had at least two phases of infection and the SkleroPro model has given good indications of when the infection events occurred.

There was no clear relationship between the total duration of infection events, the number of infection events, the timing of infection events relative to the start of flowering and stem rot incidence across all sites. Likewise mean temperature and the number of rain days during flowering did not differ significantly between high and low disease sites (data not presented).

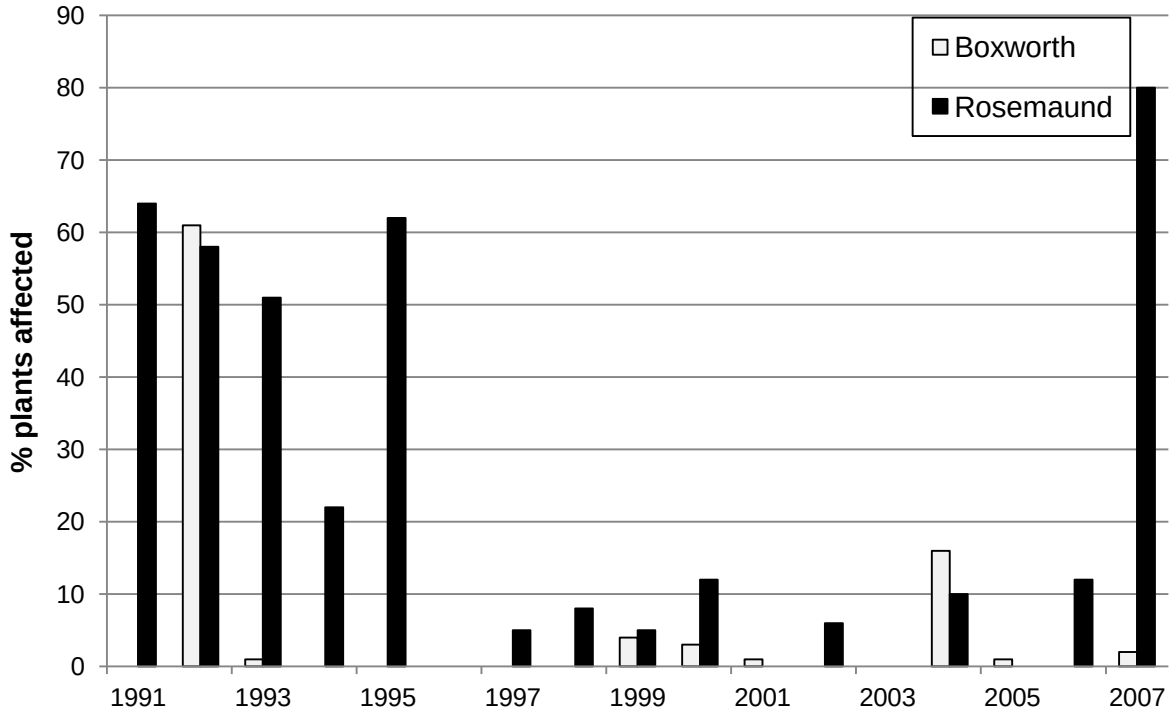

Figure 1. Mean percentage of plants with sclerotinia stem rot in winter oilseed rape crops at ADAS Boxworth and ADAS Rosemaund, 1991-2007.

Very severe attacks of stem rot have been recorded since 1991 at Rosemaund and since 1992 at Boxworth (Figure 1). Prior to this, sclerotinia incidence had been low, though there was clearly a background level of inoculum that was sufficient to generate a severe infection. At Boxworth, there was some moderately severe infection in 2004 (15% plants affected) and in 2007 most crops had 2% *Sclerotinia*, though 10% was recorded in some plots of cv. Castille. Winter oilseed rape was grown continuously between 1988 and 2002 on the field affected in 1992, but petal tests at Boxworth have indicated that inoculum levels have been low after 1992 apart from the continuous oilseed rape field in 1995, when 52.5% petals had *Sclerotinia* at early flowering. Only low levels of *Sclerotinia* inoculum have been evident at Boxworth subsequently. At Rosemaund, there have been very severe attacks (> 50% plants affected) in 1991, 1992, 1993, 1995 and 2007. In addition, stem rot affected 10-22% plants in 1994, 2000, 2004 and 2006 at Rosemaund, indicating that both inoculum and weather conditions were favourable for infection. Petal tests at Rosemaund showed high petal infection in 1994 (70% at early flowering, 97.5% at mid flowering) and in 1995 (70% at early flowering, 52.5% at mid flowering). There was lower inoculum on petals in 1996 and 1997 (25-30% petals infected) and this was higher at early flowering than at mid-flowering.

Table 1. *Sclerotinia* infection 'events' during flowering at various meteorological stations in England in 2006 and 2007.

2006			2007		
Met. Station	Region	Date of infection 'events'	Met. Station	Region	Date of infection 'events'
Andrewsfield	East	6 May	Andrewsfield	East	12 May
Andrewsfield	East	13 May	Boxworth	East	22 April
Andrewsfield	East	15 May	Boxworth	East	26 April
Boxworth	East	6 May	Boxworth	East	2 May
Boxworth	East	8 May	Boxworth	East	4 May
Boxworth	East	12 May	Boxworth	East	12 May
Boxworth	East	14 May	Boxworth	East	15 May
Boxworth	East	20 May	Brize Norton	S. east	23 April
Boxworth	East	21 May	Brize Norton	S. east	12 May
Boxworth	East	26 May	Donna Nook	East	23 April
Brize Norton	South west	7 May	Donna Nook	East	13 May
Brize Norton	South west	14 May	H'monceaux	S.east	22 April
Brize Norton	South west	16 May	Hurn	S. west	22 April
Brize Norton	South west	19 May	Hurn	S. west	8 May
Brize Norton	South west	25 May	Hurn	S. west	15 May
Coltishall	East	6 May	M.Wallop	S. east	23 April
Coltishall	East	12 May	M.Wallop	S. east	9 May
Donna Nook	East	6 May	M. Wallop	S. east	15 May
Donna Nook	East	12 May	Rosemaund	WMids	22 April
Donna Nook	East	20 May	Rosemaund	WMids	12 May
Herstmonceaux	South east	14 May	Rosemaund	WMids	16 May
Herstmonceaux	South east	17 May	Walton	East	9 May
Hurn	South west	14 May	Wattisham	East	23 April
Hurn	South west	16 May	Wattisham	East	8 May
Hurn	South west	25 May	Wattisham	East	10 May
Manston	South east	7 May	Yeovilton	S.west	23 April
Middle Wallop	South east	16 May	Yeovilton	S. west	13 May
Newcastle	North	6 May			
Newcastle	North	14 May			
Rosemaund	WMidlands	16 May			
Rosemaund	WMidlands	20 May			
Walton	East	16 May			
Wattisham	East	14 May			
Wattisham	East	16 May			
Wattisham	East	20 May			
Yeovilton	South west	14 May			
Yeovilton	South west	16 May			

Weather data at Boxworth and Rosemaund 1991-2007

The main flowering period varied in date of onset and duration during 1991-2007 (Table 2). In recent years, flowering started in early April and continued for about 6 weeks. There was later flowering of crops in 1996 and 2006 and in these years, crops were considered to be at risk during May. There were large variations in rainfall each month during the period 1991-2007, though the overall monthly average at both Boxworth and Rosemaund was about 50 mm.

At Boxworth in 1992, fungicides applied at about the mid-flowering stage on 15 May gave up to 99% control (Bowerman and Gladders, 1993). This very high efficacy suggested that all the infection had occurred soon after fungicide application. There was dry weather during 16-22 May and wet weather after this. The SkleroPro model identified infection conditions on 29 May, which has some credibility from the fungicide data. In 2004, there were four potential infection periods during 27 April-7 May at Boxworth, enabling stem rot to develop even though petal inoculum was low. Similarly in 2007, there were potentially six infection opportunities, though perhaps the events on 12 and 15 May were the most important and resulted in more disease in later flowering crops. It is perhaps surprising that there was little stem rot in 2006 when there were apparently 7 infection periods at Boxworth during flowering in May. The rainfall in May 2006 was very high (97.6 mm) and this can reduce disease risk if apothecia are flooded and petals are washed off the foliage.

A fungicide timing experiment at Rosemaund in 1992 indicated that a spray applied on the 18 May at the late flowering stage (GS 4,8) significantly reduced stem rot incidence on the main stem (c. 60% control). Untreated plots showed 66% main stems affected on 16 June and 58% plants with lesions on main stems and 34% plants with lateral stem lesions (total 84% plants affected) on 14 July. A combination of 29 April (GS 4,7) and 18 May treatments gave improved control (c. 90%) (Sansford *et al.*, 1996). SkleroPro identified infection conditions on 28 and 31 May, so mid May fungicide treatments would be expected to give good control. It is possible that late April fungicide persisted about 4 weeks and contributed to stem rot control. In 1993, at Rosemaund, sclerotinia control was rather variable, but trends suggested mid flowering sprays on 6 May gave some control, whilst late flowering sprays had little effect. SkleroPro suggested infection events might have taken place during 16-29 April and this might well explain why fungicide effects from 6 May applications were weak.

At Rosemaund in 1994, sprays applied at mid-flowering on 6 May gave up to 96% control of moderate stem rot (untreated index = 17) (Spink, 1995). Infection may have started on 5 May and this could explain some of the variation in control between fungicides because they differed in their curative activity. In 2007 at Rosemaund, there were at least two phases of sclerotinia infection judged from disease progress and fungicide performance (Ritchie *et al.*, this vol). The first infection on 22 April was identified from SkleroPro, rainfall and petal sticking records and was well controlled with fungicides applied about 11 April. The SkleroPro model identified further favourable periods for infection on 12 and 16 May. Fungicide experiments showed lower percentage control of sclerotinia than in some previous work and this could be explained by product persistence being stretched when infection occurs more than 3-4 weeks after application. As at Boxworth, more stem rot might have been expected in 2006 though there were only two infection periods and probably negative effects from very high rainfall in May (97 mm). Petal tests showed that inoculum of *S. sclerotiorum* was lower in 2006 than in 2007 (Gladders *et al.*, 2008). Previous research in England indicated that inoculum of *S. sclerotiorum* was often limiting to disease development in winter oilseed rape crops (Davies *et al.*, 1999). This is in contrast to some other areas in Europe where a high proportion of crops may be at risk.

Table 2. Dates of potential infection events* at Boxworth and Rosemaund during flowering in April and May 1991-2007.

Year	Flowering period	Rosemaund			Boxworth		
		April	May	June	April	May	June
1991	Late April -May	18 April	16 May	3			3
1992	Late April -May		28, 31 May	2	30 April	29 May	2
1993	midApril-midMay	16, 22, 29 April		3	28 April	2	1
1994	midApril-midMay		5 May	2		6 May	
1995	midApril-midMay	24 April		2			4
1996	May		22 May	1		22, 23, 26,28 May	
1997	April-midMay	25,30 April	15 May	4	25, 26 April	15 May	4
1998	April-midMay	1 April	11 May	3	1, 21 April	11 May	9
1999	April-midMay	3, 20, 25 April	6 May		3, 20 April	1	1
2000	April-midMay			1	27 April	8 May	1
2001	midApril-midMay		13 May			13 May	2
2002	April-midMay		6, 12 May	2		8, 12 May	1
2003	midApril-midMay	24 April	16 May			16 May	2
2004	April-midMay	16, 27, 29 April		1	27, 29 April	2, 7 May	2
2005	April-midMay	25 April		1	25, 29 April	3 May	4
2006	May		16,20 May,			6, 8, 12, 14, 20, 21, 26 May	
2007	April-midMay	22 April	12, 16 May		22,26 April	2, 4, 12, 15 May	7

*Infection events defined as > 23 hours, > 7 °C and 80% RH

SkleroPro is a forecasting model for sclerotinia stem rot that has been validated against historic data in Germany. (Koch *et al.*, 2006). In the UK, it appears to be useful for identifying infection periods in the UK. It is not certain that it has identified all infection periods as stem rot was reported near to Brize Norton and Coltishall that had no infection events in 2007. Local variation in rainfall and relative humidity may be responsible for such discrepancies.

Unfortunately, fungicides have little curative activity and should be applied **before** a risk period has occurred. The usefulness of the SkleroPro model for guiding decision making will therefore depend on how reliably periods of high humidity can be forecast. Currently, spray applications for stem rot control should take into account forecast rainfall so that treatment is made before petals are likely to stick to the foliage.

An unusual feature of the epidemics in 2006 in Scotland and 2007 in England was the late development of stem rot during June, suggesting that there may have been survival of inoculum and plant infection at or after the end of flowering possibly via senescing leaves (Gladders *et al.*, 2008). The SkleroPro model identified infection periods in mid-May suggesting that infection probably took place at the late flowering stage. The stem symptoms would have appeared in early June and this would be consistent with low disease incidence at some sites in late May. Infection at late flowering also occurred in 2008 and fungicides applied at early flowering showed limited persistence and did not give good control of this late infection (Ritchie *et al.*, this volume). The benefits of a second fungicide spray at the late flowering stage were not determined in 2007, but a two-spray programme is likely to be cost-effective where yield loss is expected to be more than 10%.

However, even in stem rot prone areas there are large annual variations in disease risk (Koch *et al.*, 2006). This situation may be changing in the UK as more oilseed rape crops have been grown and shorter rotations are used. The south-west has shown higher stem rot infection than other regions in previous years and 2007 problems may be due to sclerotial inoculum produced in 2005. The consequences of the 2007 and 2008 epidemics may be greater production of ascospore inoculum and increased disease risk for several years.

There is now be greater awareness of the risk of yield loss from late flowering infection and the benefits of maintaining protection with fungicides during the whole flowering period. The forecasting of infection periods and better quantification of inoculum of *S. sclerotiorum* in individual crops are priorities for future research.

Acknowledgements

Funding from the Home-Grown Cereals Authority for this review is gratefully acknowledged.

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Evaluation of a decision making tool for controlling *Sclerotinia* stem rot in WOSR

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Abstract: *Sclerotinia* stem rot is a major disease of oilseed rape in France. Severe yield losses usually occur two to three times per decade. For controlling the disease by spraying a fungicide only when it is needed CETIOM has improved and evaluated a diagnostic test based on a measured proportion of oilseed rape flowers infected by *Sclerotinia sclerotiorum.* The score of this test is used as a risk indicator. Receiver operating characteristic (ROC) curves are used to determine a decision threshold according to the sensitivity and specificity values. The threshold of 30% infected flowers generally leads to correct decisions except when disease pressure was too high, as in 2007.

Key words: oilseed rape, *Sclerotinia sclerotiorum*, decision making means

Introduction

Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a major disease on winter oilseed rape in France. The disease incidence varies one year to the next and generally severe yield losses occur only two or three times per decade. Until the late nineties control of the disease was performed by spraying a single preventive fungicide during flowering. But this practice has led to a large number of unnecessary sprays and the emergence of resistance to currently used fungicides (Penaud *et al.*, 2003). In order to reduce the application of chemicals and its negative impact on the environment, a reliable decision making tool for disease management is required.

Previous studies were carried out to define risk indicators for the control of *Sclerotinia* stem rot based on a petal test (Turkington & Morrall, 1991) or check-list (Maisonneuve *et al.*, 1997). An improvement of the petal test was made by replacing the medium used to a more selective one and by plating flowers instead of petals under field conditions (Taverne *et al.*, 2003). A comparison of these different risk indicators suggested the use of the percentage of infected flowers as the more convenient (Makowski *et al.*, 2005). This risk indicator was studied at field level for nine years and Receiver Operating Characteristic (ROC) curves were used to evaluate the performance of this indicator test for decision making.

Material and methods

For 9 years, a set of field experiments were carried out by CETIOM with partners from extension services located in different French regions where rapeseed is grown.

Rapeseed growers were requested to leave a 12 m by 20-30 m plot unsprayed. In this plot, the farmer or the technician of his extension service performed a diagnostic test at early flowering by collecting 40 flowers from 40 different plants in four sampling sites. Flowers were plated on 9 cm diameter Petri dishes filled with a semi-selective agar medium. The Petri

dishes were incubated at 22-23 °C for 4 days before each petal was scored for the presence of a colony of *S. sclerotiorum* (in this case, the blue coloured medium turned yellow) and the percentage of infected flowers was calculated. Disease incidence was assessed at the end of the growing season, 3 to 4 weeks before harvest.

For 9 years a total of 830 field plots were available for analysis. A ROC (receiver operating characteristic) curve analysis was done to compare the annual performance of diagnostic test. The 830 field plots were divided into two subgroups depending on a disease incidence threshold (D_{thresh}) of 10% diseased plants which represent an injury level above which a treatment was needed. Each indicator value (I) was compared to another threshold I_{thresh} which represented a decision threshold above which treatment was recommended. The results were used to calculate the true positive rate (TP) and the true negative rate (TN). TP was the number of plots with $I > I_{thresh}$ in the subgroup of plots with $D > D_{thresh}$ divided by the total number of plots in that subgroup and represented correct decisions to spray divided by the total number of fields that needed spraying. TP is referred to as "sensitivity". TN was the number of plots with $I \leq I_{thresh}$ in the subgroup of plots with $D \leq D_{thresh}$ divided by the total number of plots in that subgroup and corresponded with correct decisions to not spray divided by total number of fields that should not be sprayed. TN is referred to as "specificity". ROC curves plot the sensitivity (TP) versus (1-specificity) at all possible decision thresholds. A ROC curve that passes close to the point (0.1) shows that the indicator has both desirable sensitivity and specificity characteristics. The diagonal line is the no-discrimination line. The accuracy of the indicators was estimated using the area under the curve (AUC).

Results

Between 2000 and 2008, the percentage of infected flowers showed annually a great variability (Figure 1), lowest in 2003 with a mean score of 21% and highest the last 5 years with a mean 60% of infected flowers.

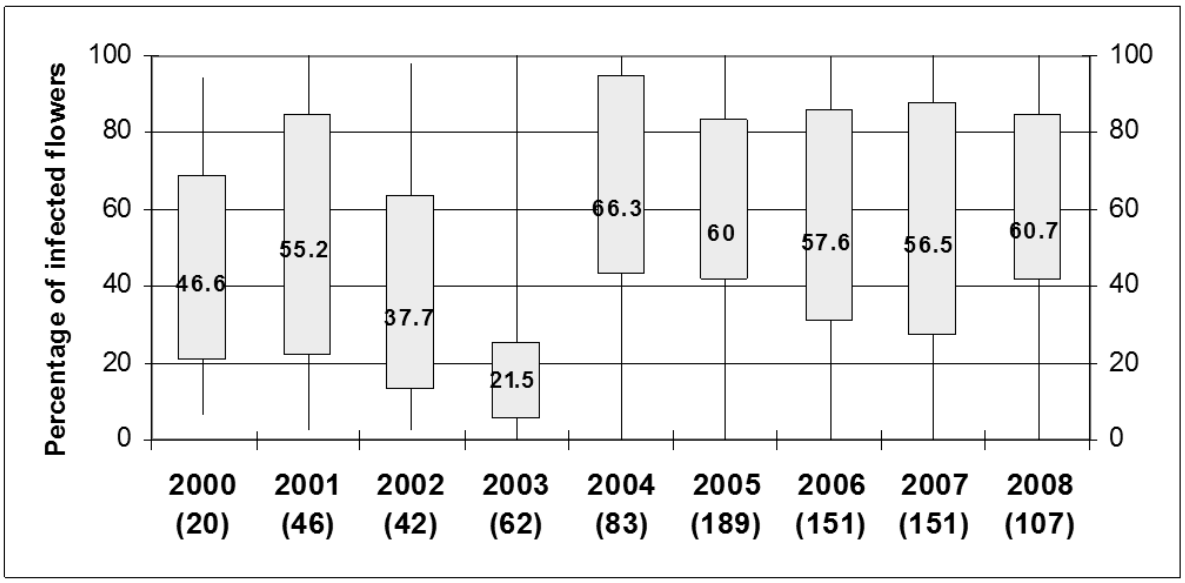

Figure 1. Annual distribution of the percentage of infected flowers assessed at flowering 2000-2008.

The disease incidence also showed some variability (Figure 2). Whereas, a low disease incidence occurred in 2001, 2003, 2004 and 2005, a moderate incidence was encountered in 2000, 2002 and 2006. The most severe epidemic was in 2007. In 2008, the mean disease incidence was moderate except in Champagne region where the epidemic was as severe as the previous year. A similar value of risk indicator corresponded to a different disease incidence, i.e. in 2004 and 2007. There was no direct relationship between the risk indicator and the disease incidence.

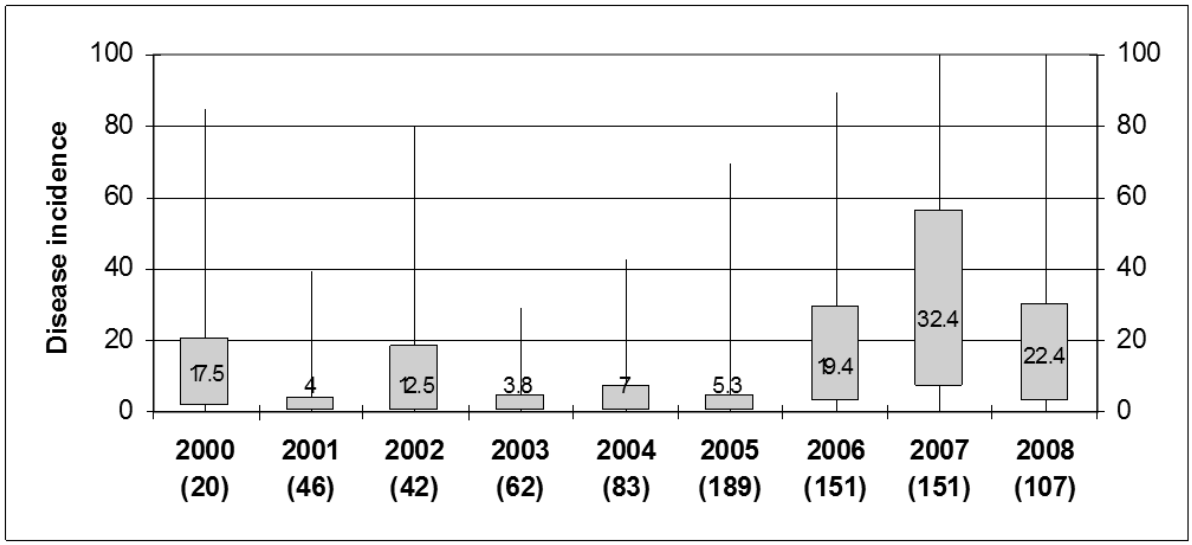

Figure 2. Annual distribution of the disease incidence from 2000 until 2008

However, for 9 years, 65% of field plots show little or no disease with $D_{thresh} < 10\%$ whereas 35% of field plots indicated more than of 10% of diseased plants (Figure 3). At a decision threshold $I \leq 30\%$, the false negative given by the percentage of fields that did not need spraying but reached more than 10% of diseased plants (upper left quarter) was limited to 3.6%.

The use of ROC analysis allowed us to assess the annual performance of the risk indicator (Figure 4). For 9 years, seven ROC curves were positively above the "no discrimination" line that suggested the risk indicator was informative. In contrast, the risk indicator failed to be informative in the years 2001 and 2005, where the disease pressure was low. In conditions of severe disease pressure in 2007 the AUC of the risk indicator was not significantly different from the mean AUC for the 9 years taken as a whole.

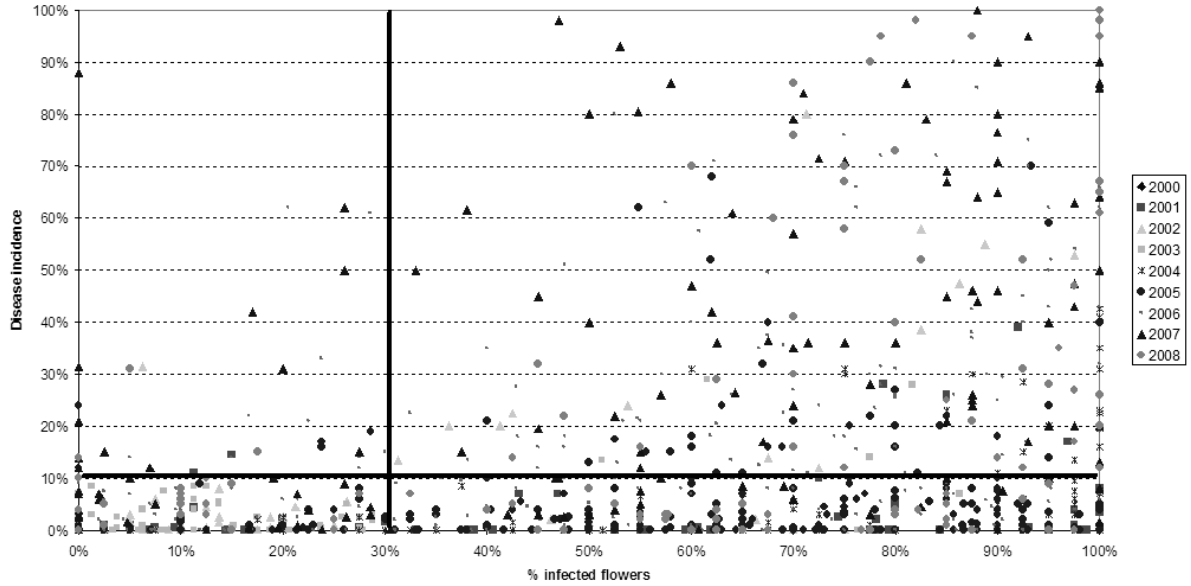

Figure 3. Relationship between risk indicator (percentage of infected flowers) and final disesase incidence from 830 fields during the period 2000-2008.

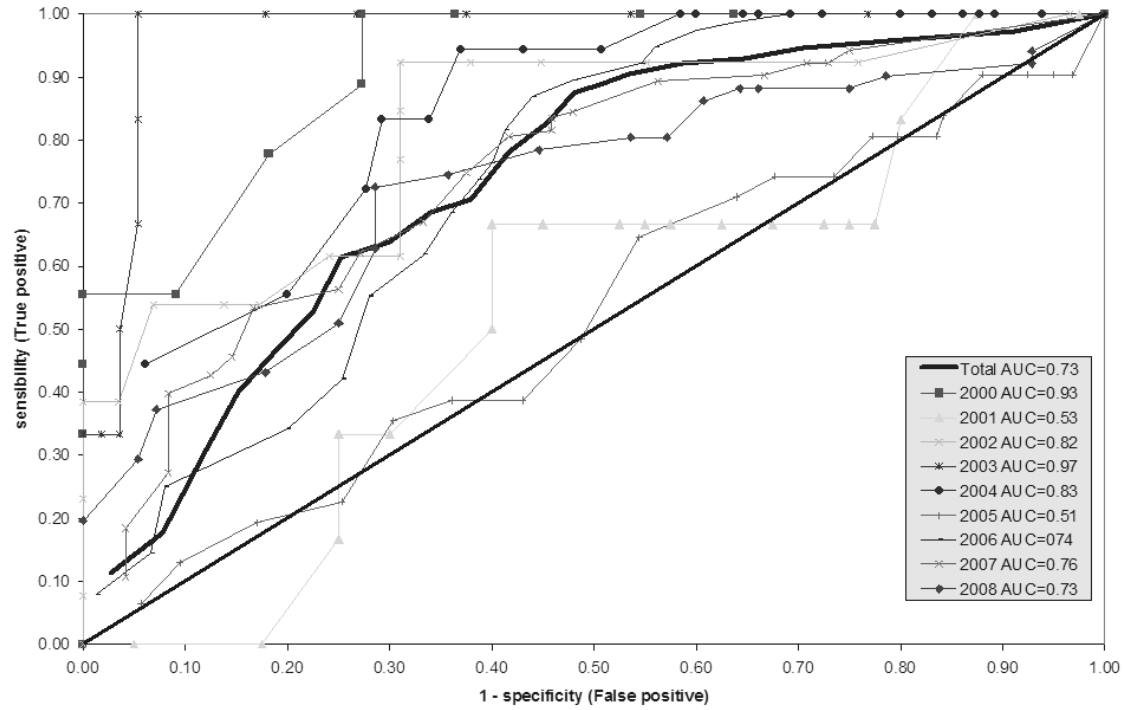

Figure 4. ROC curves for 9 years at disease incidence threshold of 10% of diseased plants.

Our objective was also to avoid the false negative decisions. That leads to consider 1-sensitivity in function of the possible values of the decision threshold. Figure 5 can be used to select a decision threshold according to the risk of false negative. If the rate of false negative was 10%, then the decision threshold corresponded to 30% that can be considered as a good compromise solution. By reducing the false negative to 5%, the decision threshold would be

15%. If any false negative was required, then no threshold could be defined; that also meant that no spray could be avoided and farmers continue to apply systematic treatments.

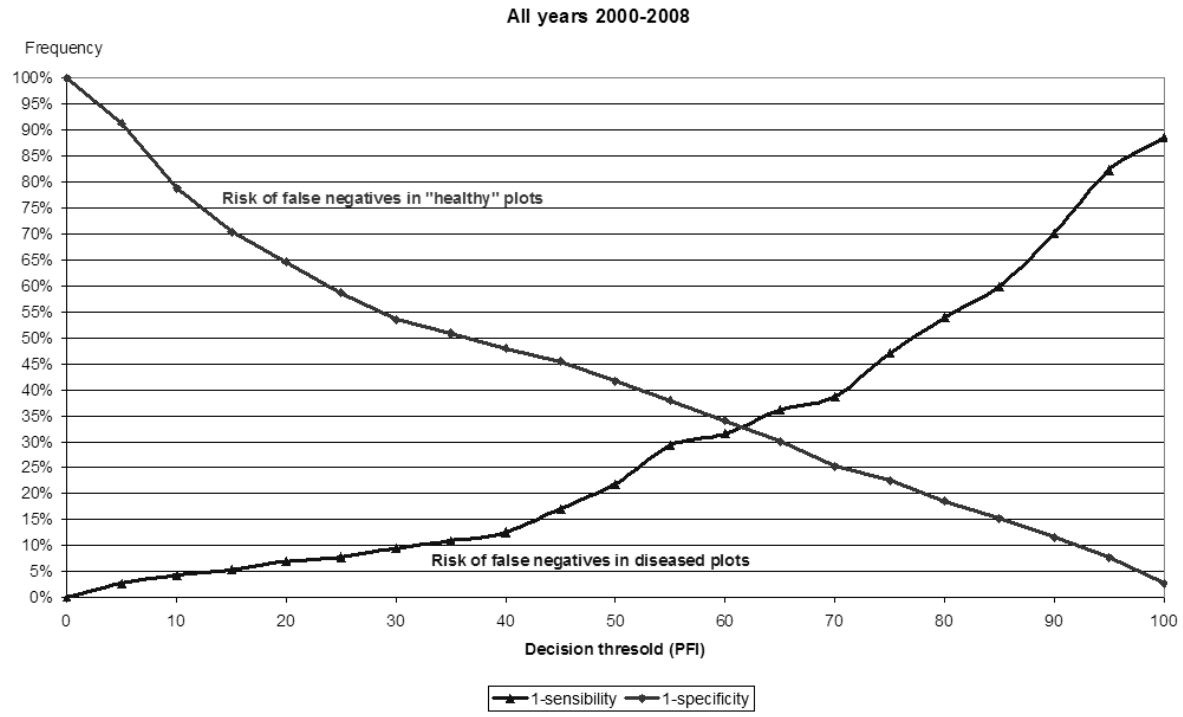

Figure 5. 1-sensitivity and specificity values given for all possible decision thresholds.

Discussion

The risk indicator designed by the percentage of infected flowers was not perfect. Because the risk was estimated at the early beginning of flowering, it does not take into account the development of the disease after the flowering stage. Previous studies on such risk indicators have already indicated this limit depending mainly on weather conditions (Turkington *et al.*, 1991, Penaud *et al.*, 2006). A long term meteorological forecast should improve the decision making.

Up to now farmers feel more or less reluctant to use the risk indicator for decision making because of false negative decisions. Different ways to improve the performance of the indicator could be investigated (i) by lowering the decision threshold but that means an increase of unnecessary sprays of fungicide, (ii) by repeating the diagnostic test 8-10 days later to better take into account an increase of the primary inoculum or (iii) by developing an insurance system. At last the decision making could also be improved by combining the risk indicator expressed at field level and climatic models.

Moreover, the detection of colonies *of S. sclerotiorum* on Petri dishes is sometimes confusing. So, it could be convient to use a specific method such as real time PCR (Ruck *et al.*, 2007). Studies are in progress to replace the threshold of infected flowers by a reliable PCR threshold.

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Monitoring the sensitivity of *Sclerotinia sclerotiorum* to fungicides

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Abstract: *Sclerotinia sclerotiorum* isolates from oilseed rape are monitored every year for fungicide resistance. In the past, the use of carbendazim was very popular and led to a widespread resistance among *S. sclerotiorum* populations, especially in the oilseed rape cropping areas. Regarding dicarboximides (*eg* iprodione, vinclozoline or procymidone), very few resistant isolates have been detected in several French regions, not affecting field efficacy. These fungicides are no longer registered in France. For two years, new fungicides belonging to carboxamides and the DMI families have been registered to control this disease. Thus, determining *S. sclerotiorum* baseline sensitivity to these new compounds was necessary to manage sustainable use. A method based on mycelial growth on synthetic agar medium amended with a concentration of 2 mg/l was developed for boscalid, prothioconazole and metconazole. The methods allowed us to determine the sensitivity to boscalid of around 1400 field isolates; no resistance to boscalid was detected in 2007. The method was less reliable for DMI compounds.

Key words: *Sclerotinia sclerotiorum*, fungicide resistance, carboxamides, DMI fungicides

Introduction

Sclerotinia stem rot is one of the major diseases of oilseed rape that can cause severe damage and important yield losses. The control of the disease is based on the application of fungicides using a preventive and systematic spray at the beginning of flowering.

The wide use of the same active ingredient for more than fifteen years has led to the emergence of resistant strains of *Sclerotinia sclerotiorum* to carbendazim belonging to benzimidazole fungicides (Souliac *et al.*, 1995). Since the first detection of resistant strains, a monitoring programme has been carried out every year in a network that involves SPV as the national crop protection service, INRA BIOGER as scientific support and CETIOM as the technical center for oilseed crops. Recently, chemical companies were also involved.

In the beginning, the monitoring only took into account carbendazim (MBC) and then dicarboximide fungicides (Penaud *et al.*, 2003). As these fungicides failed to be re-registered, monitoring has moved to cover the new registered compounds such as boscalid and prothioconazole. In this paper, the main results of the *Sclerotinia* monitoring are presented.

Material and methods

Samples of 20-25 sclerotia of *S. sclerotiorum* were collected in oilseed rape fields in different locations in France.

From each sample, 20 isolates of *S. sclerotiorum* were obtained by plating 10 half-sclerotia on 2% malt extract agar + 15 mg/l streptomycin. From the margin of 5-days- old colonies, mycelium plugs of 6 mm diameter were removed to test the sensitivity of these isolates based on inhibition of mycelial growth on fungicide-amended or unamended media. Cultures were incubated at 20 °C in the dark for 3 days and then mean colony diameters were measured.

For the sensitivity test to MBC and dicarboximide fungicides, malt extract agar medium was amended with 1 and 10 mg/l carbendazim and 1 and 2 mg/l iprodione using commercial formulations (Bavistine DF and Kidan, respectively). Isolates were considered as sensitive when mycelium did not grow at the two concentrations.

For the sensitivity test to boscalid and DMI, a minimal medium using Na succinate as a carbon source was amended with a final concentration of 2 mg a.i./l of boscalid, metconazole and prothioconazole, respectively. Each technical product was previously dissolved in ethanol. Control plates were amended only with ethanol. In both fungicide amended or unamended media, ethanol did not exceed 0.5%. When the radial growth was less than twice the diameter of the mycelial plug, the isolates were considered as sensitive. When the radial growth was superior to 50%, the isolate was considered as resistant. An intermediate radial growth of between 25% and 50% required a repeat test with a range of concentrations 0, 0.5, 1, 2 and 5 mg/l.

Results

Sclerotinia monitoring during 2000-2005 was carried out to follow the spread of resistance to carbendazim. Around 1400 field samples were tested. Between 2000 and 2004, the resistance to carbendazim occurred mainly in north-eastern and central regions where 60% to 70% of monitored locations exhibited resistant isolates to carbendazim (Figure 1). A year later in 2005, all field samples collected in Lorraine were found to be resistant to carbendazim. In the other regions, the resistance to carbendazim was detected in 75% of the monitored fields. Due to the widespread of carbendazim resistance and the restricted use of this fungicide to 250 g/l until 31/12/09, there was no more interest to continue MBC monitoring.

For 6 years (2001-2006) of sclerotinia monitoring to dicarboximide fungicides, less than 1% of isolates, i.e. 9 locations, were detected as resistant to iprodione (Figure 2). These isolates exhibited a low fitness *in vitro*. Moreover no lack of efficacy was observed in the field.

In 2007 *Sclerotinia* monitoring was focused on the newly registered compounds boscalid and prothioconazole (Figure 3).

For boscalid, the method based on radial growth of sclerotinia colony on minimal medium with succinate as a carbon source was assessed as a reliable method. And among 114 field samples, no resistant isolate was detected.

For DMI, the method using one discriminant concentration of prothioconazole or metconazole has often allowed some restricted growth of aerial mycelium. So the method was not as direct as the previous ones and in many cases it was required to test a range of concentrations, especially for metconazole. After this extended check, all isolates were finally assessed to be sensitive.

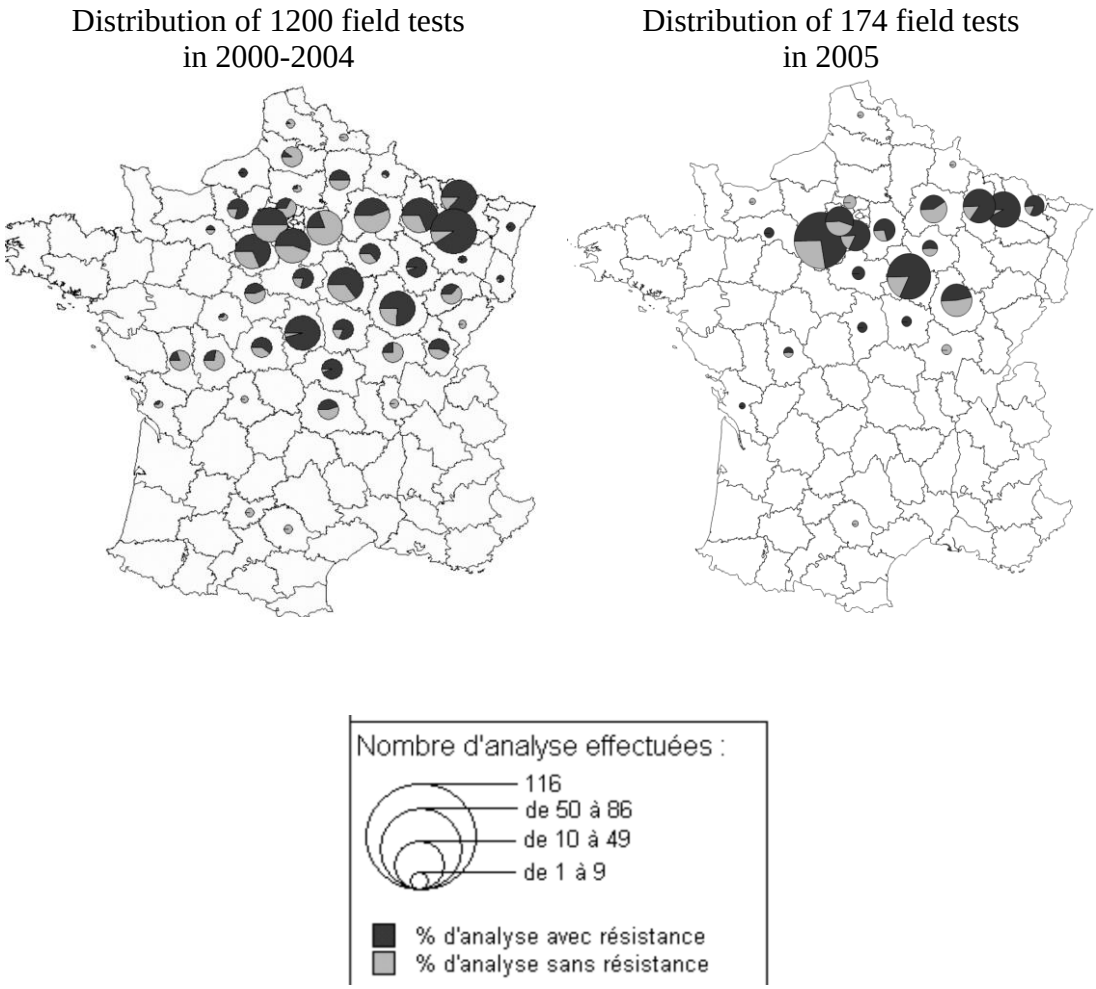

Figure 1. Proportion of carbendazim resistant *S. sclerotiorum* isolates. (The size of the circle indicates the number of *S. sclerotiorum* isolates tested, dark part of the circle indicates the % of resistant isolates).

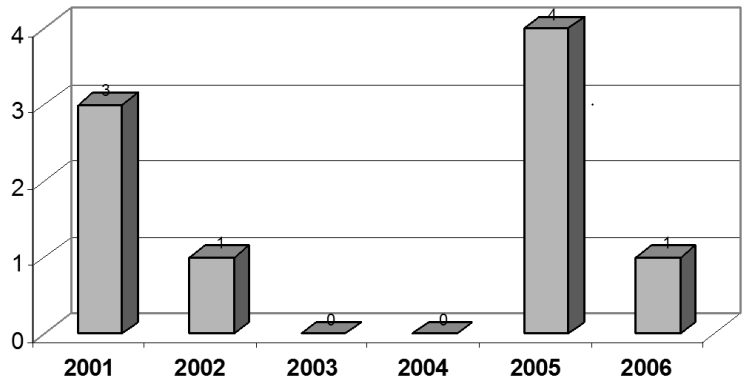

Figure 2. Occurence of isolates of *S. sclerotiorum* resistant to iprodione during 2001-2006. Shown are numbers of fields with at least one isolate resistant to iprodione.

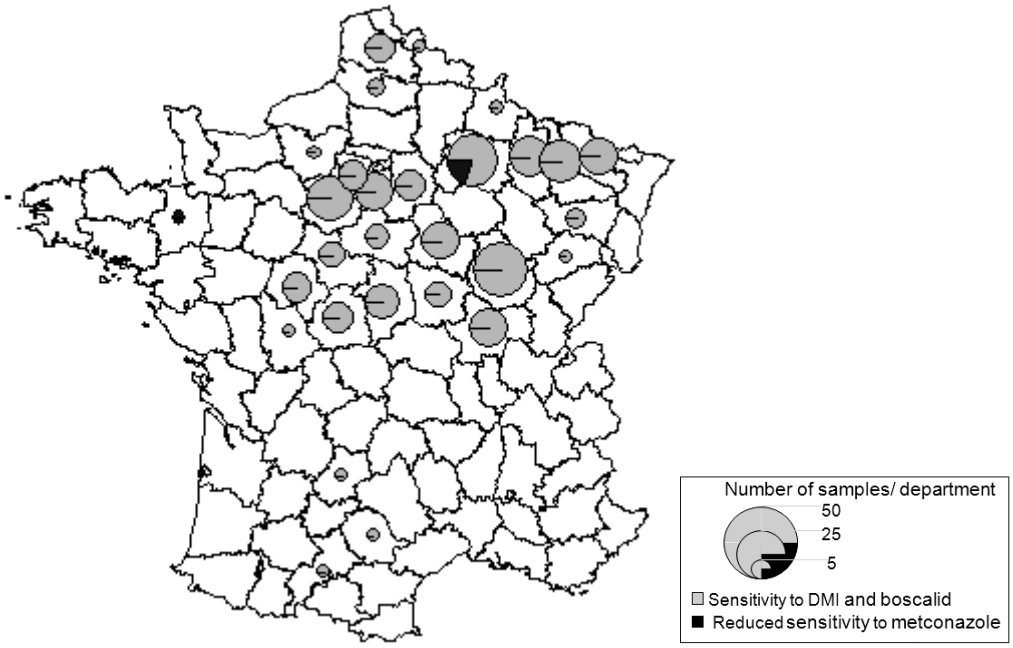

Figure 3. Distribution of 114 fields monitored for sensitivity to boscalid and DMI in 2007

Discussion

Resistance of *S. sclerotiorum* to benzimidazole fungicides is present everywhere in French oilseed rape fields. Since the emergence of the first isolates resistant to carbendazim in 1994 in Burgundy (Souliac & Leroux, 1995), the development of widespread MBC resistance in sclerotonia took a decade. A previous analysis of cultural practices suggested that the development of resistance to carbendazim was associated with short rotations of oilseed rape (every 2 or 3 years) and at least 5 sprays of carbendazim during the last ten years (Penaud *et al.*, 2001). In spite of low levels of *Sclerotinia* stem rot, the benzimidazole resistance has remained in the areas where it was previously detected suggesting that resistance of *S. sclerotiorum* to carbendazim is stable.

Alternatively, iprodione resistant isolates were detected in few fields suggesting they could have emerged by chance in the local population of *S. sclerotiorum*. Moreover a low fitness could explain that iprodione resistant isolates could fail to remain in the wild population.

With neither MBC or the dicarboximide fungicides being re-registered, chemical control has changed to use new fungicides. At the moment fungicides registered to control sclerotinia stem rot belong to 3 groups: carboxamide fungicides with boscalid, DMI with metconazole, tebuconazole, cyproconazole or prothioconazole and QoI with azoxystrobin. Because these are single-target fungicides and therefore associated with a moderate to high risk for developing resistance, a monitoring programme is justified. Firstly, sclerotinia monitoring has been focused on boscalid and DMI because these fungicides are widely used. But it could also be enlarged to QoIs.

Although no boscalid-resistant isolate was found after 3 years of intense use, strategies to reduce the risk of fungicide resistance are needed. Every year recommendations are indicated to farmers:

- Use crop rotation including less susceptible crops;

- Reduce soil infestation by using biological agents such as *Coniothyrium minitans;*
- Use fungicides for *Sclerotinia* control according to *Sclerotinia* risk identified by a forecasting system;
- When it is needed, spray at the optimum timing from the appearance of the first pods;
- Avoid repeated use of the same active ingredient in the same field.

Alternate use of effective fungicides only when application is necessary could delay the development of resistance and make possible a durable crop protection of oilseed rape.

Acknowledgments

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Fungicide application timing and control of *Sclerotinia* stem rot in England in 2007 and 2008

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Abstract: Stem rot (*Sclerotinia sclerotiorum*) on winter oilseed rape has caused significant yield losses in the UK in 2007 and 2008. Industry-funded trials in the West Midlands investigated the effect of fungicides on stem rot control in 2007 and 2008. These trials demonstrated that the onset of infection and fungicide application timing to be significant factors in stem rot control in oilseed rape. In 2007, initial infection occurred in late April/early May whereas infection occurred in mid to late May in 2008. Fungicides applied in late April gave good early control of the epidemic in 2007. In contrast, in 2008, fungicides applied in late April did not control the epidemic as effectively as those applied in early May due to the later onset of the epidemic. In both years, the control of stem rot infection declined as the season progressed following a single mid-flower fungicide application. Strong fungicide protection persisted for about three weeks. The significance of these results on *Sclerotinia* control is discussed.

Key words: *Sclerotinia sclerotiorum*, stem rot, oilseed rape, fungicides

Introduction

Sclerotinia sclerotiorum is a widespread and destructive fungal pathogen and is known to colonise over 400 plant species worldwide (Bolton *et al*., 2006), including oilseed rape (*Brassica napus* ssp. *oleifera*). Stem rot is present in UK oilseed rape crops every year, however, it was particularly severe during 2007 and 2008, when 36% and 35% of crops were affected respectively (www.cropmonitor.co.uk). With almost 600,000 ha (Anon., 2008) oilseed rape grown in the UK in 2008, this represents a significant threat to future cropping.

Most infections in the UK are caused by sclerotia germinating carpogenically in spring to produce apothecia. These release ascospores into the air which land on leaves, stems, petals and pods. Ascospores can also infect neighbouring crops and have been shown to be capable of travelling up to 150 m (Williams & Stelfox, 1979). During flowering, petals stick to leaves and act as a nutrient source for the pathogen (Davies, 1986) as well as transferring ascospores onto green tissues. The pathogen needs a series of specific weather events to establish itself in a crop including warm, wet soil conditions for sclerotial germination, dry conditions during ascospore release and rainfall to cause petals to stick to leaves and stems. Once plants have become infected, the pathogen produces sclerotia, predominately inside the stems of infected plants (Paul & Rawlinson, 1992). Although there has been recent research into the resistance of cultivars to stem rot (Bradley & Khot, 2006), currently there are no commercially available resistant cultivars. Fungicides are therefore an important component of disease control strategies. They have little curative activity and therefore fungicide application timing is critical. It was apparent in 2007 and 2008 that single fungicide sprays gave poor control of stem rot where they were applied early in the season and there was a late epidemic of stem rot. This paper examines the performance of a standard fungicide, boscalid, in various field

experiments in 2007 and 2008 in relation to the onset of epidemics and fungicide application timing on the control of *Sclerotinia* stem rot.

Material and methods

Replicated field trials were established in the Hereford area, West Midlands in September 2007 and 2008 (Table 1) in commercially grown crops of oilseed rape on a farm with a history of *Sclerotinia* stem rot infection. Fungicides were applied at early to mid-flower as shown in Table 1. Natural *Sclerotinia* infection developed in all trials. Monitoring of the development of the disease epidemic throughout the growing season was done by assessing 25 plants for stem rot symptoms from across the trial sites from early flowering onwards in both 2007 and 2008. *Sclerotinia* incidence (% plants infected) and severity (0 to 4 index) was assessed post-flowering and pre-harvest in all trials. Stem rot incidence was recorded for 100 plants per plot and severity of these lesions categorised using a 0 to 4 index (0 = no disease; 4 = plant dead). Results were converted into a 0 to 100 disease index for each plot. Selected boscalid treatments [as Filan (50% w/w boscalid), BASF plc] and the effect on disease control and yield (adjusted to 91% dry matter) data are presented in this paper.

Table 1. Sites for *Sclerotinia* experiments in 2007 and 2008.

Trial (year)	Location	Date sown; harvested	Cultivar	Application date (growth stage)
1 (BASF2007)	Weobley, Herefordshire	1 September 2006; 3 August 2007	Catalina	10 April (4,3)
2 (BASF2007)	Weobley, Herefordshire	1 September 2006; 3 August 2007	Catalina	19 April (4,5)
3 (HGCA2007)	Weobley, Herefordshire	1 September; 3 August 2007	Catalina	11 April (4,3)
4 (BASF2008)	Weobley, Herefordshire	4 September 2007; 14 August 2008	Castille	22 April (4,5)
5 (HGCA2008)	Weobley, Herefordshire	2 September; 15 August	Castille	1 May (4,5)

Results and discussion

Stem rot infection in 2007 and 2008

Flowering of winter oilseed rape occurred from early April until mid-May in 2007 and throughout April and May in 2008. In both 2007 and 2008, disease pressure for *Sclerotinia* stem rot at the trial sites was very high (Figure 1). In 2007, petal stick in crops was obvious by 23 April and *Sclerotinia* stem rot was established at the trial site by 12 May, with 18% incidence of small (index 1) lesions in untreated plots across the trial site (Figure 1). The minimum criteria for stem rot infection events of 23 hours at 7 °C followed by > 80% relative humidity (as defined by SkleroPro: Koch *et al.*, 2006) was used in 2007 to determine when infection events had occurred (Gladders *et al.*, 2008). These were 22 April, 12 and 16 May

and the first date coincided with the onset of petal stick. In contrast in 2008, petal stick was obvious by 2 May, with the first lesion found in untreated plots 11 days later on 13 May. The incidence and severity of stem rot increased rapidly, with 68% infection two weeks later on 28 May and 96% plants affected 8 days later on 6 June. The minimum criteria for stem rot development as defined by SkleroPro were met on seven occasions during flowering in 2008: 20 and 29 April, and 15, 16, 26-28 May.

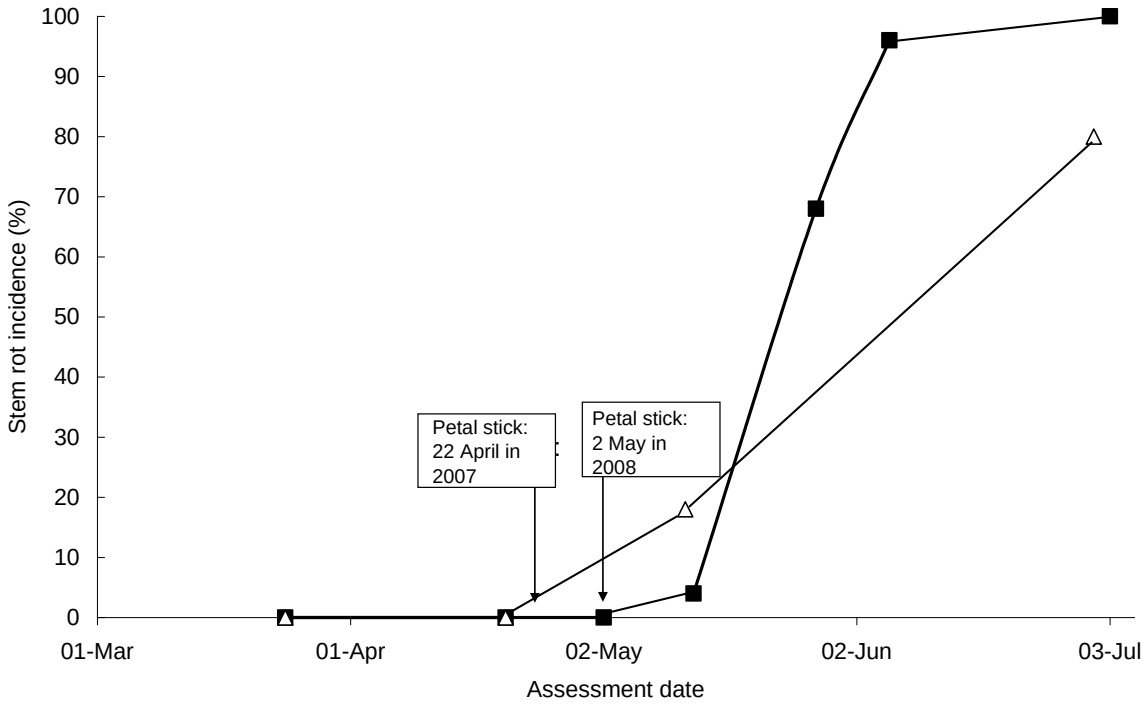

Figure 1. Progress of *Sclerotinia* stem rot epidemic in 2007 (Δ) and 2008 (■).

Sclerotinia stem rot control in 2007

In 2007, fungicides were applied 10 days apart with trial 1 sprayed on 10 April and trial 2 sprayed on 19 April (Table 1). At the early post-flowering assessment in June, *Sclerotinia* stem rot incidence in both trials was significantly reduced by fungicide application to between 2.5 and 3.8% compared to 24.5% and 33.0% in untreated plots (Table 2). By 30 June, incidence in untreated plots had increased to between 61 and 68% and plots treated with fungicide had 12 and 13.5% respectively. As significant levels of infection were detected in untreated plots by 12 May (18%), the first infection would have occurred two to three weeks earlier, coinciding with the infection event on 22 April. Boscalid gave good control (c. 90%) in both experiments on 1 June, but this had declined to c. 80% control on 30 June, suggesting that persistence of fungicide control had started to decline (Table 2).

In 2007, yield response to *Sclerotinia* stem rot control was between 1.66 and 2.29 t/ha in trial 3 (Figure 2). Lower incidence and severity of *Sclerotinia* stem rot at full rate resulted in a significant yield increase of 0.5 t/ha compared to that achieved by quarter rate. Stem rot control was about 75% at 0.75 and full dose and comparable to trial 1 that was sprayed one day earlier.

Table 2. Disease incidence and severity of *Sclerotinia* stem rot in 2007

	Trial 1 (BASF2007)			Trial 2 (BASF2007)		
	1 June	30 June		1 June	30 June	
Treatment	% inc	% inc	severity	% inc	% inc	severity
Untreated	24.5	61.3	56.1	33.0	68.0	67.3
Boscalid 250g a.i.	2.5	13.5	9.1	3.8	12.0	11.3
FPr	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SED (48 df)	3.15	4.12	4.31	2.99	3.92	3.74
LSD @ 5%	6.33	8.29	8.67	6.02	7.89	7.54

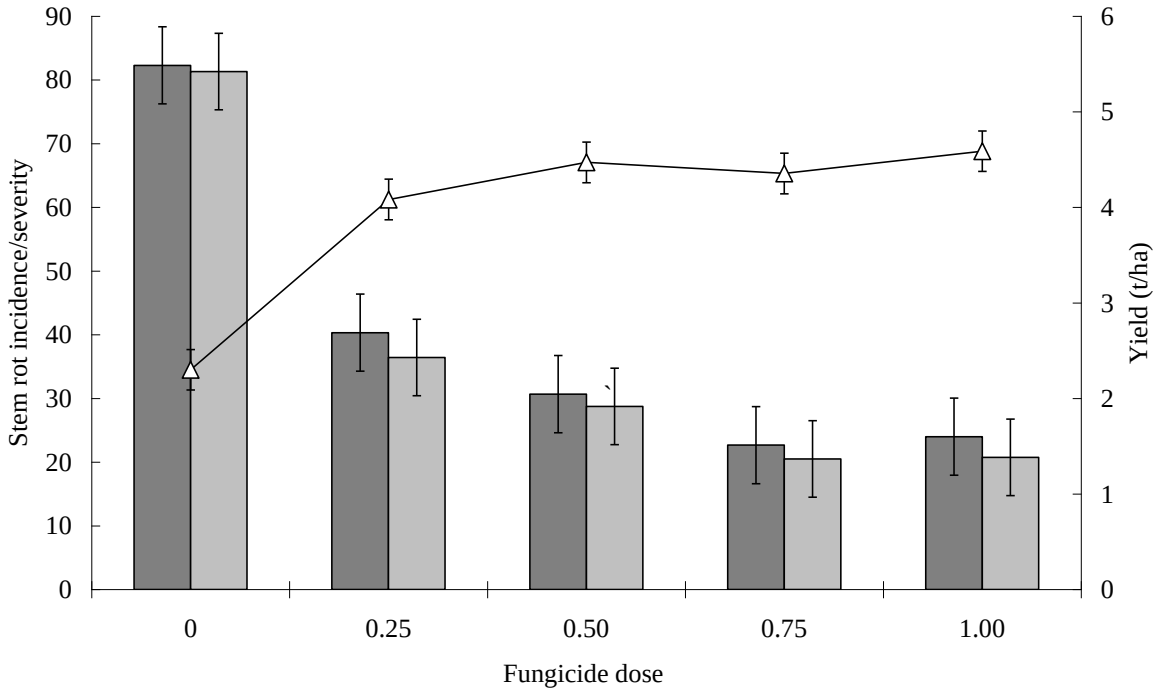

Figure 2. Effect of fungicide dose on *Sclerotinia* incidence (■), severity (■) and yields (Δ: t/ha) in 2007 in trial 3 (HGCA2007). Lines represent the LSD @ 5% (P = 0.005). Full dose of boscalid was 250 g a.i./ha.

Sclerotinia stem rot control in 2008

Fungicides were applied 9 days apart on 22 April in trial 4 and 1 May in trial 5 (Table 1) and these experiments were in the same field. By 6 June, both trials had a similar incidence (31-35%) and severity (19.4-20.4) of *Sclerotinia* stem rot in untreated plots. However, trial 4 was showing *Sclerotinia* stem rot symptoms in fungicide treated plots and 80% control, whereas there was no stem rot in treated plots in trial 5 (Table 3). An assessment 19 days later on 25 June showed that *Sclerotinia* control based on stem rot incidence decrease to 29% in trial 4 and to 57% in trial 5 (Table 3).

In 2008, the first stem rot lesions were seen on 13 May (Figure 1), so initial infection occurred during late April/early May, probably on 29 April, when an infection event occurred. The persistence of fungicide treatment in trial 4, which had a 22 April application, had declined very sharply about four weeks following application and had not controlled late stem rot infection as effectively as those applied on 1 May. There were two late infection events on 15 and 16 May, and fungicide application 3.5 weeks prior to them on 22 April did not protect from late infection, whereas 1 May application, 2.5 weeks prior to these infection events, showed no stem rot symptoms on 6 June (Table 3). By 25 June, stem rot incidence and severity had increased further, with late April/early May fungicides unable to protect against further infection events at the end of May. In trial 5, disease incidence increased from 35 to 78% in untreated plots and from 0 to 34% in treated plots, indicating only 20% control of late stem rot. Thus, strong fungicide persistence was only three weeks.

Table 3. Disease incidence and severity of *Sclerotinia* stem rot in 2008.

	Trial 4 (BASF2008)				Trial 5 (HGCA2008)			
	6 June		25 June		6 June		25 June	
Treatment	% inc	severity	% inc	severity	% inc	severity	% inc	severity
Untreated	31.3	20.4	42.0	28.8	35.0	19.4	78.0	44.3
Boscalid 250g a.i.	6.3	3.1	29.8	24.5	0	0	33.7	13.2
FPr	< 0.001	< 0.001	0.032	0.046	< 0.001	< 0.001	< 0.001	< 0.001
SED (45 df)	5.13	6.12	7.08	14.23	2.64	1.044	4.20	2.95
LSD @ 5%	10.34	3.04	14.27	7.07	7.33	2.899	8.42	5.90

***Sclerotinia* control and yield**

In 2008, there was > 2.0 t/ha yield response to fungicide application at mid-flower (Figure 3). There were small reductions in disease incidence as fungicide dose increased, but no significant differences between yields for treated plots.

In these experiments in 2007 and 2008, boscalid application at mid-flower significantly improved yields by 1.79-2.19 t/ha and 1.66-2.29 t/ha, respectively, compared to untreated plots (Table 4). At current prices, a response of only 0.2 t/ha is required to cover the cost of the fungicide and its application. As stem rot was only partially controlled, there was potential to increase yields with additional fungicide treatments.

Conclusions

The data presented here demonstrated differences in the development of *Sclerotinia* stem rot epidemics and in disease control from year to year. The earlier onset of the epidemic in 2007 meant that control by a single fungicide application at the standard early to mid flowering timing (mid April) was sufficient to protect the crop until the end of flowering. In 2008, there was later onset of the epidemic and the occurrence of a second phase of infection in mid-May meant a single application in late April was not an effective strategy. The single early May applications did not protect crop against infection at the end of flowering in late May. In 2008

there was an extended flowering period of at least 8 weeks in many crops and at least two fungicide applications will be required to protect the crop against stem rot with this scenario. In both years, mid-flower fungicide application for the control of *Sclerotinia* stem rot prevented yield loss of between 37% to 47% in 2007 and 47% to 49% in 2008 regardless of timing of fungicide application. The persistence of fungicides appears to be limited to about three weeks after application, thereafter efficacy of disease control will decline. A second fungicide treatment may well have improved disease control and yield responses. When stem rot is expected to cause losses > 0.5 t/ha, two fungicide applications are likely to be very cost effective. The onset and duration of the disease epidemic and timing of fungicide applications are clearly important factors to consider when determining the best strategy for controlling stem rot on oilseed rape. The application of a second fungicide should be considered for high risk situations about three weeks after the first treatment.

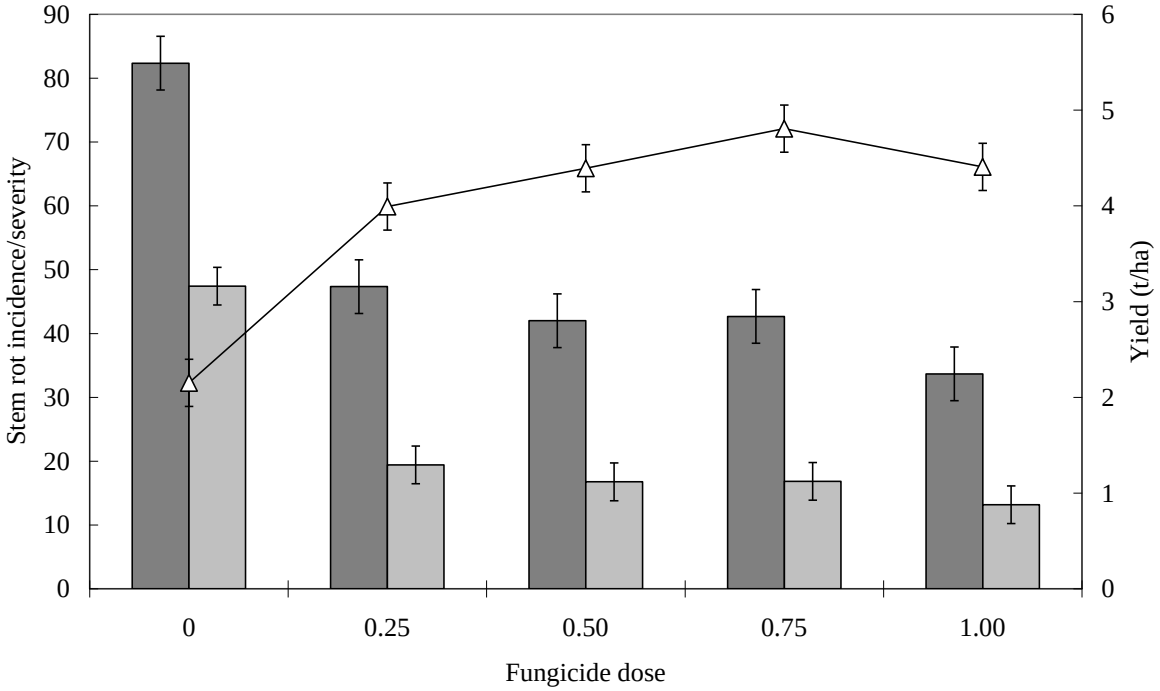

Figure 3. Effect of fungicide dose on *Sclerotinia* incidence (■), severity (■) and yields (Δ: t/ha) in 2008 in trial 5 (HGCA2008). Lines represent the LSD @ 5% (P = 0.005). Full dose of boscalid was 250 g a.i./ha.

Table 4. Yield response to boscalid application at mid-flower in 2007 and 2008.

Year	Trial	Treatment	Yield (t/ha)	Yield response (t/ha)
2007	1 (BASF 2007)	Untreated	2.95	-
		boscalid 250g a.i	4.69	1.74
		FPr	< 0.001	
		SED (48 df)	0.136	
		LSD	0.273	
	2 (BASF 2007)	Untreated	2.40	-
		boscalid 250g a.i	4.48	2.08
		FPr	< 0.001	
		SED (48 df)	0.145	
		LSD	0.292	
	3 (HGCA 2007)	Untreated	2.30	-
		boscalid 250g a.i	4.59	2.19
		FPr	< 0.001	
		SED (48 df)	0.212	
		LSD	0.425	
2008	4 (BASF 2008)	Untreated	1.71	-
		boscalid 250g a.i	3.39	1.66
		FPr	< 0.001	
		SED (45 df)	0.240	
		LSD	0.484	
	5 (HGCA 2008)	Untreated	2.10	-
		boscalid 250g a.i	4.00	2.29
		FPr	< 0.001	
		SED (58 df)	0.246	
		LSD	0.492	

Acknowledgements

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