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 the

Centre de Recherche Public – Gabriel Lippmann, Belvaux, Luxembourg

8th – 10th October, 2013

Edited by

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IOBC-WPRS Bulletin
Bulletin OILB-SROP Vol. 104, 2014

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

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

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

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ISBN 978-92-9067-285-2 Web: http://www.iobc-wprs.org

Darmstadt, 2014

Preface

This meeting was held 8th - 10th October 2013 at the Centre de Recherche Public, Gabriel Lippmann, Esch/Belvaux, Luxembourg. This meeting was extremely well organized locally by Michael Eickermann and colleagues. Thank you very much for your hard work -a great job done!

In total 55 participants from 14 countries and 27 different institutions attended for the meeting. Participants originated from Belgium, Czech Republic, Denmark, Estonia, France, Germany, Hungary, Luxembourg, the Netherlands, Nigeria, Poland, Sweden, Switzerland and the United Kingdom. Altogether 43 papers (28 oral & 15 posters) were presented during the three days of our meeting. The nice and friendly atmosphere stimulated intense scientific exchange. Altogether we consider this as a very successful meeting.

For the first time awards for the 'best poster' and 'best young scientist presentations' were given. These awards were donated by the Center de Recherche Publique, Gabriel Lippmann, which we gratefully acknowledge. "The Best Entomology Poster Award" was given to Henrike Hennies and Bernd Ulber, who presented work on screening for resistance to cabbage root fly. The "Best Pathology Poster Award" was adjudicated Xavier Pinochet and co-workers who presented their study on the durability of Rlm7 stem canker resistance. Alicja Gronowska, Helle Mathiasen, Riina Kaasik, Ivan Juran and Daniel Lopisso received the "Young Scientist Best Presentation Awards".

Our conference dinner at the Wine Cellar "Bernard-Massard" at Grevenmacher was preceded by a guided tour of the winery where we learned how the wine is made using the 'traditional method'. We have never seen so many bottles (4 million bottles are leaving the cellars annually!!!), high volume tanks and stainless steel at a single location. It was extremely fascinating!

After a small wine tasting session we had a very delicious sushi-style conference dinner within the winery. A wonderful social event! Many thanks again to Michael who selected this amazing place and to the people from Bernard-Massard, who are responsible for this remarkable event which we will fondly remember!

During the meeting the Convenor successor to Birger Koopmann was elected. There were two candidates standing for election: Prof. Malgorzata Jedryczka (IGR-PAS, Poznan, Poland) was elected. She accepted the vote and is now in the primary Convenor of the Working Group for the next five years (Sam remains the co-convenor). Gosia, we are sure you will do a great job and we wish you all the best for this duty!

Finally, we successfully laid plans for organizing the next Working Group meeting. Eve Veromann agreed to take responsibility for the local organization at the University of Life Sciences in Tartu, Estonia. Thank you very much Eve! We are sure this is a fascinating venue and we will have a very interesting meeting. We like to invite all of you – and your colleagues working in this area – to participate in this meeting. Due the 14th International GCRIC Rapeseed Congress, which will be held in Canada 2015, we decided to switch the date of the meeting to October 2016, which in consequence modulates the biannual character of our meetings. However ....

... head ja turvalist reisi ja kohtumiseni Eestis, Tartus!

Sam & Birger

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Current diseases of winter oilseed rape worldwide and their control

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Abstract: Increased areas of oilseed rape in Europe – and worldwide are resulting from demand of the common market for plant oil and farmers' response to high profitability of this crop. This has had a high impact on the pest and diseases pressure of the crop. High intensity of oilseed rape cultivation has resulted in large monocultures (block cropping) and reduced rotations, which highly increase the risk of disease epidemics. The literature list several groups of diseases of winter oilseed rape: seed-borne, soil-borne, foliar-base, viruses and phytoplasms. Their correct identification and knowledge on their spread is crucial, as it determines the best control strategy. Disease symptoms caused by most phytopathogenic fungi are easy to recognise by experts with good quality training. Low expertise may result in misidentification of the causal agent, and this may lead to the use of unnecessary or even counterproductive control measures, such as the use of improper biological and chemical treatments. The lack of sufficient knowledge makes it possible to confuse plant infection with harmful microorganisms, insect pest damage or lack of nutrients. The most important diseases worldwide and in Europe are phoma leaf spot and stem canker, *Sclerotinia* stem rot or white mould, black spot and clubroot. The first three are caused by phytopathogenic fungi, whereas clubroot is caused by a protist. In some regions grey mould and/or *Verticillium* has been observed. Plants of oilseed rape are also commonly attacked by powdery and downy mildews. It is less common to encounter light leaf spot or *Fusarium*. Many of the diseases occurring at the very start of seed germination and cotyledon development are well controlled by seed treatments. The control of numerous diseases strongly depends on the accuracy of pathogen detection at genus or species level. Current techniques of molecular biology allow quick identification, not only at species level, but also in relation to chemotype, pathogenicity group, the allele of avirulence gene, race composition and resistance status to specific fungicides. Beside early detection and identification, current strategies of integrated pest management also include forecasting the occurrence of different stages of a pathogen, such as mature fruiting bodies, spores or other forms of inoculum. Current decision support systems include real-time detection of the pathogen (eg. SPEC, www.spec.edu.pl) or weather and pathogen biology based mathematical models (eg. Blackleg Sporacle, SimMat, SIPPOM, SimAsco). Research on biological control of oilseed rape pathogens is under way.

Key words: fungal diseases of oilseed rape, integrated pest management, decision support systems

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Impact of regional climate change on pest insects in oilseed rape

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Abstract: Global and regional climate models are suitable tools to simulate the future climate. However, climate change projections are afflicted with different uncertainties, e.g. due to an incomplete coverage of all physical processes involved. A possible way to deal with these uncertainties related to model results is to analyze an ensemble of equally valid, possible realizations of regional climate model projections. Furthermore, the use of an ensemble of future climate projections leads to more accurate estimates of the potential future changes, because the effect of the internal variability can be more accurately addressed. Examples of multi model ensemble approaches in agriculture are discussed in the literature. Aside from effects on agricultural practice, possible impacts of global climate change are also expected on pest species in crop production, e.g. species distribution or shifting of crop invasion. Since 2009, an expert group of meteorologists, entomologists and modellers has been investigating the effect of regional climate change on pest species in oilseed crops for Luxembourg within the framework of several research projects. So far, projections have been done for the rape stem weevil, *Ceutorhynchus napi* (Gyll.), the cabbage stem weevil, *Ceutorhynchus pallidactylus* (Mrsh.) and the pod midge, *Dasineura brassicae* (Winn.). Based on an ensemble of 6 regional climate change projections, the bandwidths of possible change signals and the uncertainty associated with these projections were investigated for the near (2021 - 2050) and far future (2069 - 2098) in comparison to a reference time period (1961 - 1990). All projections were based on the Special Report on Emissions (SRES) A1B emission scenario of the Intergovernmental Panel on Climate Change (IPCC). This scenario describes anthropogenic emissions of a future world with rapid economic growth, an increasing global population until the middle of this century and a balanced use of fossil and non-fossil energy resources.

Key words: crop invasion, ensemble projection, impact study, rape stem weevil, regional climate change

Introduction

Regional climate models (RCM) are suitable tools to simulate the future climate, but their projections are afflicted with different uncertainties, e.g. due to an incomplete coverage of all physical processes involved (Knutti *et al.*, 2010). One way to deal with these uncertainties related to model results is to analyze an ensemble of equally valid, possible realizations of regional climate change projections. So far, ensemble-based analyzes have rarely been used to investigate possible impacts of climate change on agriculture (Juroszek & Tiedemann, 2013). Aside from effects on agricultural practice, probable impacts of global change are also expected on pest species (Junk *et al.*, 2012) in agricultural crop production.

The rape stem weevil, *C. napi*, is widely distributed in Central Europe (Alford *et al.*, 2003) and also a common pest species in Luxembourg (Braunert, 2009). Adults immigrate into crops of oilseed rape from their overwintering habitats in the soil of last year’s crop in early springtime (Alford *et al.*, 2003). Females of *C. napi* oviposite individual eggs into the top of the main stems or lateral racemes close to the growing tip of the plant (Williams, 2010). The larvae mine within the pith of host plants. Mature larvae migrate into the soil for pupation. Adults develop after 4-5 weeks, but remain in the cocoon until emergence in the following spring (Alford *et al.*, 2003). As resistant cultivars are not available (Eickermann & Ulber, 2010), information about the first activity of this pest species in early springtime is necessary for the exact timing of insecticide application (Petraitienė *et al.*, 2012). The objective of this study was to project the impact of regional climate change conditions on the period of the emergence of the rape stem weevil from the soil in early spring in the region of Luxembourg. We combined the results of regional climate change models originating from the EU FP6 ENSEMBLES project (www.ensembles-eu.org) with a statistical model for predicting the emergence of *C. napi*.

Material and methods

A selection of six regional climate change projections from the EU FP6 ENSEMBLES project (van der Linden & Mitchell, 2009) was used. This selection was based on three criteria: i) simulation results with a 25 km × 25 km spatial resolution, ii) a temporal coverage up to 2098, and iii) the overall ensemble bandwidth in terms of air temperature change signals must be covered by the selected RCMs in order to account for many different possible future climate evolutions. The time-span up to the year 2000 is considered as the 'control time-span' (based on observed 20th century greenhouse gas emission data-sets) followed by the 'projected time-span' (based on the Intergovernmental Panel on Climate Change (IPCC), Special Report on Emissions Scenarios (SRES) A1B emission scenario (IPCC 2007). As the Hadley Centre Coupled Model, version 3 (HadCM3)-driven RCMs temporal coverage ends in December 2098, the far-future time-span is defined from 2069 until 2098 and the near-future time-span is defined from 2021 until 2050. Variables retrieved for this study are minimum, maximum and mean daily air temperatures as well as daily totals of precipitation. These variables were used to derive soil temperatures according to a method presented by Junk *et al*. (2012). Because RCM outputs are systematically biased in comparison to observations, it was necessary to bias-correct them before using them with a threshold-based impact model (Piani *et al.*, 2010). A linear bias correction approach was used to correct air temperature (for further details see Goergen *et al.* (2013)). The range of projected changes in future air temperature are comparable with the results presented in the new 'Fifth Assessment Report: Climate Change 2013` by the IPCC (IPCC 2013).

We chose a threshold-based statistical relationship between soil temperature (50 mm depth) and the day of the year (DOY) of emergence of *C. napi* from the soil according to Oppermann (1990). The emergence takes place when a daily maximum soil temperature of 6 °C has been reached for the first time in the year (Oppermann, 1990).

The applied Kolmogorow-Smirnow-Test indicates that all time series of the predicted DOY show a non-Gaussian distribution. Therefore the Mann-Whitney U-Test (SigmaStat Ver. 3.0, Erkrath, Germany) was used to test for statistical significant differences between the projected emergence periods for the different time-spans.

Results and discussion

Based on a multi-model average, a spatial mean air temperature for Luxembourg is given in Figure 1 for the time span from 1961 until 2098 (thick grey line). The ensemble bandwidth is expressed by +/-1 x standard deviation (grey shading). In addition, the long-term (30 years) mean values for the three analyzed time-spans are given (dashed lines). An increase of the annual air temperature by 1.2 °C (near future) to 2.8 °C (far future) can be expected in comparison to the reference time period (1961 - 1990). In contrast to this statistical significant increase in the annual mean near surface air temperatures, the annual precipitation totals do not show any clear trends. A more detailed analysis of the monthly precipitation sums indicates a shift towards wetter winters and dryer summers in the area under investigation (data not shown here).

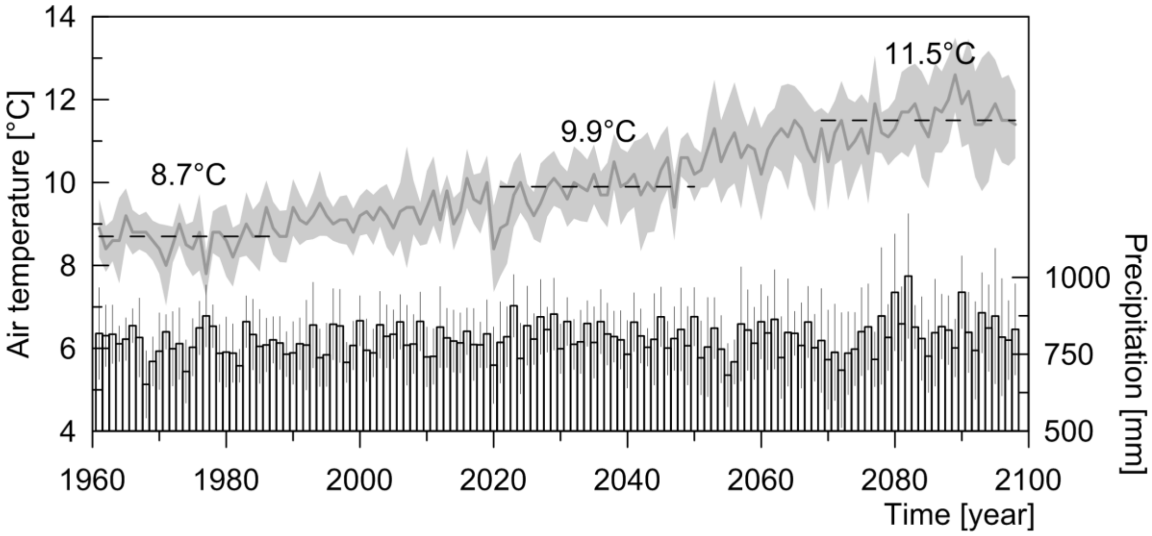

Figure 1. Time-series of annual mean air temperature (spatial mean for Luxembourg): multi model (n = 6) spread (grey shading); multi model mean (thick grey line) with long-term annual means from 1961 to 1990, 2021 to 2050, and 2069 to 2098 (dashed lines). Annual precipitation sums are given as bar charts in the lower part of the figure: vertical lines represent +/-1 x standard deviation of the annual values (derived from daily totals).

The results of the combination of the climate change projections and the statistical model by Oppermann (1990) are presented as absolute frequency distributions for three different time-spans: the reference period (1961-1990), the near future (2021-2050) and far future (2069-2098) time-spans. Each frequency distribution consists of the annual event, expressed as day of the year, for each year and model, leading to 180 elements per frequency distribution (30 years for each of the six models).

Using the threshold-based model of Oppermann (1990), statistical significant differences between the three time-spans for the emergence of *C. napi* in crops of oilseed rape can be observed (Figure 2). In the control time-span, emergence occurred on average at DOY 101 (11 April). For the two future time-spans, shifts towards earlier dates are predicted: DOY 82 (23 March) for the near and DOY 65 (6 March) for the far future (Figure 2). It is well known that increasing air temperatures can shorten the diapause (Bale & Hayward, 2010) and may lead to earlier activity of insect species (Harrington *et al.*, 2001). A similar effect was detected for the cabbage seed weevil, *C. obstrictus*, for the cabbage stem weevil, *C. pallidactylus* (Junk *et al.*, 2012) and for several butterfly species on brassicaceous host plants (Roy & Sparks, 2000).

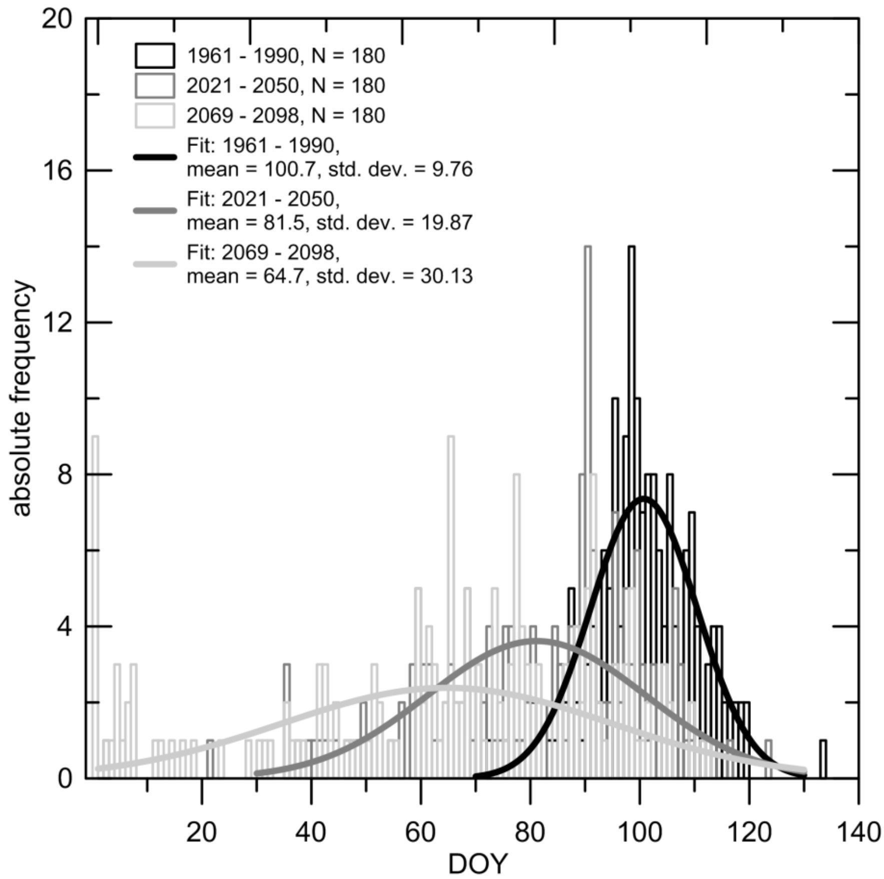

Figure 2. Absolute frequency of the day of the year (DOY) for the emergence of *C. napi* from fields grown with oilseed rape in the previous year (according to the model of Oppermann (1990); threshold: mean daily soil temperature of 6 °C at a depth of 50 mm) derived from 30 year time-spans and 6 RCMs (N = 180, all available data combined into a single distribution). Gaussian fits are given for the time-spans from 1961-1990, 2021-2150, and 2069-2098 to simplify interpretation. Arithmetic means and the standard deviations of the distribution functions are also given.

The time-span in which the potential crop invasion will take place based on the average minimum and maximum value of each of the models increased from 61 days in the reference period, to 121 in the near, and 112 in the far future. The elongation of this period is mainly caused by an earlier onset while the maximum values show only slight variations. A longer flight duration under increasing air temperature was also shown for numerous butterfly species in the analysis of long-term datasets in UK (Roy & Sparks, 2000). The projected prolongation of the period of emergence of *C. napi* will increase the risk of missing the appropriate time-frame for an insecticide application (Harrington *et al.*, 2001).

Acknowledgements

We gratefully acknowledge the financial support of the Ministère de l’Enseignement Supérieur et de la Recherche (MESR) of the Grand Duchy of Luxembourg in the framework of the REMOD project.

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IOBC-WPRS Bulletin Vol. 104, 2014
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Quantification of ecological services for sustainable agriculture

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Abstract: The project aims to identify the key semi-natural habitats (SNH), outside and within crops, providing essential ecological services (ES). Vegetation traits will be linked to potential ES provision, case studies will measure actual ES levels and inform models which will show unused opportunities and trade-offs among ES by SNH from habitat to landscape scale. This will be achieved for a range of representative cropping systems and farming intensities in regions dominated by agriculture and matched to the requirements of local and national stakeholders. Surveys will identify key SNH and existing literature will be used to link their vegetation traits to ES provision. ES provision will be measured in existing habitat types (SNH to crop) across economically important cropping systems, farming intensities and four European agro-climatic zones using simple techniques in 16 case studies. A case study is defined by a unique combination of region, crop species, and service. Each case study will concentrate on locally important cropping system and the main ES required. Pollination and pest control have been identified as main ES needed, but also soil fertility, weed control and social services will be considered. The relative socio-economic weight of the studied ecosystem services will be appraised using feedback from national experts using a semi-quantitative method. Data will parameterise spatially explicit models to determine how the vegetation composition, management, shape, area, and placement of SNH in agricultural landscapes affect the distribution of mobile-agent based ecosystem services from farm to landscape level. To investigate synergies and trade-offs in ecological services, multi-criteria analysis will be developed to combine a suite of modules in an integrative modelling framework. Outputs are designed to inform local, national and EU stakeholders on how to improve ES provision from SNH and will include a novel web-based tool.

Key words: semi-natural habitats, trade-off, landscape, cropping system, agro-climatic zone

Funding: FP7-KBBE-2012, Collaborative project
Project coordinator: PhD. John Holland, Game and Wildlife Conservation Trust, UK.
The QuESSA project is funded by the European Commission through the Seventh Framework Programme. Contract Number 311879

SEVENTH FRAMEWORK PROGRAMME

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IOBC-WPRS Bulletin Vol. 104, 2014
pp. 11-15

Oilseed crops in the Czech Republic and their health state in 2013

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Abstract: In the Czech Republic the following crops are grown as oilseeds: winter oilseed rape (*Brassica napus*), white mustard (*Sinapis alba*), sunflower (*Helianthus annuus*), poppy (*Papaver somniferum*), brown mustard (*Brassica juncea*), oilseed flax (*Linum usitatissimum*), and on a small area soybean (*Glycine soja*) and peanut pumpkins (*Cucurbita oleifera*). Areas of oilseeds increase every year particularly the proportion of winter oilseed rape. This year's rapeseed area reached 401.3 thousand hectares. Among the most important fungal diseases of oilseeds are *Phoma* stem canker (*Phoma lingam*) and white rot of oilseed rape (*Sclerotinia sclerotiorum*). Among the important pests of oilseeds are stem and pod weevils (*Ceutorhynchus* spp.), pollen beetle (*Meligethes aeneus*) and Brassica pod midge (*Dasyneura brassicae*). The second most important oil plant in the Czech Republic is sunflower with 24.6 thousand hectares grown in 2013. The most significant diseases and pests of sunflower include gray mold (*Botrytis cinerea*), sunflower white rot (*Sclerotinia sclerotiorum*) and aphids (*Brachycaudus helichrysi* and *Aphis fabae*). Poppy takes third place of the most commonly grown oilseeds with 18.36 thousand hectares grown in 2013. Downey mildew (*Peronospora arborescens*), leaf blight (*Pleospora papaveracea* and *Dendryphion penicillatum*) and occasionally gray mold (*Botrytis cinerea*) have been reported as problems. Mustard was cultivated in the Czech Republic at 16.94 thousand ha. Among the major diseases of white mustard include white rot (*Sclerotinia sclerotiorum*), *Alternaria* leaf spot of *Brassica* (*Alternaria* spp.) and gray mold of crucifers (*Botrytis cinerea*).

Key words: Czech Republic, oilseeds, oilseed rape, sunflower, poppy, mustard, diseases, pests

Introduction

In the Czech Republic winter oilseed rape (*Brassica napus*), white mustard (*Sinapis alba*), sunflower (*Helianthus annuus*), poppy (*Papaver somniferum*), brown mustard (*Brassica juncea*), oilseed flax (*Linum usitatissimum*), and on selected fields soya (*Glycine soja*) and peanut pumpkins (*Cucurbita oleifera*) are grown as oilseeds (CSO, 2013).

Areas of oilseeds increase every year, the most significant increase has been in the proportion of winter oilseed rape, which in 2013 reached 401.3 thousand ha. The average yield for the last three years was 2.73 t/ha. The total production in 2012 was 1,109 thousand tons of rapeseed (CSO, 2013). Rapeseed contains 35-40% oil. From one hectare of oilseed rape we get about 3 tons of oil seeds, which are squeezed to get approximately 1,100 kg of rapeseed oil, from which 70 thousand tons of methyl esters were produced as additives for fuels (Laurin, 2008). Rapeseed oil is very low in saturated fatty acids and high in monounsaturated and essential fatty acids, omega-6 and omega-3 (Suchý *et al.*, 2008). In the Czech Republic the winter form of oilseed rape is primarily grown; in 2013, spring oilseed rape (*Brassica napus* convar. *napus* f. *annua*) occupied about 2% of the sown area (Zeman & Volf, 2012). Organisations in the *Association Czech Rape* are engaged in breeding Czech varieties of winter oilseed rape. The most recent registered varieties include Oponent (2006), Oksana, Opus, Aplaus (2007), Benefit (2009), Cortes, Orion, Oceánie (2012) and Rescator (2013) (Štěpánek, 2013).

Among the most important fungal diseases of oilseeds include *Phoma* stem canker (*Phoma lingam*) and white rot of oilseed rape (*Sclerotinia sclerotiorum*). Another fungal disease *Peronospora parasitica* does not currently cause serious problems. The importance of clubroot (*Plasmodiophora brassicae*) is primarily local in contaminated areas and is exacerbated by a combination of short rotations, early sowing and the retention of oilseed volunteer plants. Among the important pests of oilseed rape include stem and pod weevils (*Ceutorynchus* spp.), pollen beetle (*Meligethes aeneus*) and Brassica pod midge (*Dasyneura brassicae*) (Kazda, 2013 a, b). In the Czech Republic an increased level of tolerance of pollen beetle to pyrethroids was found (Seidenglanz *et al.*, 2012). In recent years damage to plants (roots) has been caused by cabbage root flies (*Delia radicum*) (Seidenglanz *et al.*, 2013).

The second most important oil plant in the Czech Republic is sunflower. In 2012 sunflower was cultivated on 24.6 thousand ha.; production amounted to 56.9 thousand tons of seeds, with the average yield for the last three years amounting to the equivalent of 2.3 tons/ha (CSO, 2013). Sunflower seeds contain on average 45% of oil. The sunflower oil is commonly used as edible oil, for frying and deep-frying, as well as a moisturizing ingredient in cosmetics (Suchý *et al.*, 2008). The most significant adverse biotic factors of sunflower cultivation in the Czech Republic include gray mold (*Botrytis cinerea*), sunflower white rot (*Sclerotinia sclerotiorum*), and aphids (*Brachycaudus helichrysi* and *Aphis fabae*) (Veverka & Vyvadilová, 2013).

Poppy is the third most commonly grown oilseed crop in the Czech Republic. It is grown mainly as a food crop. Poppy straw (capsules and upper part of the poppy stem) is also used in pharmacology. In 2012 poppy was cultivated on 18.36 thousand ha; harvested seed production amounted to 12.8 thousand tons. The average yield for the last three years was 0.67 t/ha (CSO, 2013). Poppy seeds contain 45-50% oil. Poppy seed oil is easily digestible, contains large amounts of vitamin E and has no narcotic effects. Poppy seed contains particularly high doses of tocopherols other than vitamin E (α-tocopherol). Other uses include in the manufacture of paints, varnishes and soap; poppy oil is also used in medical radiology. (Pennington & Douglass, 2005). Among the major diseases of poppy in the Czech Republic include poppy downey mildew (*Peronospora arborescens*), leaf blight of poppy (*Dendryphiella* sp., *Dendryphion penicillatum*), gray mold of poppy (*Botrytis cinerea*), *Alternaria* spot of poppy and blacks on capsules (*Pleospora herbarum*) (Kazda & Prokinová, 2009).

Mustard was cultivated in the Czech Republic in 2012 on 16.94 thousand ha; production was 15.46 thousand tons of seeds. The average seed yield for the last three years was 0.81 tons/ha (CSO, 2013). Among the major diseases of white mustard are *Sclerotinia* white rot (*Sclerotinia sclerotiorum*), *Alternaria* leaf spot *(Alternaria* spp.) and gray mold of crucifers (*Botrytis cinerea*). *Sclerotinia sclerotiorum* sclerotia impairs the quality of the harvest especially in seed crops intended for the food industry. Incidence of the disease is particularly prevalent following heavy rainfall during ripening (Plachká, 2013).

Material and methods

Field monitoring

To describe the current situation of crop health in Czech fields, we monitored selected oilseed crops for pests and diseases all over the country in cooperation with farmers in the 2012/2013 season. To evaluate the pest and disease pressure, we used appropriate EPPO standards.

Weather conditions
Meteorological data were derived from websites of the Czech Hydrometeorological Institute.

Summary data
Data on planting area, production and yield in previous years were derived from websites of the Czech Statistical Office, public databases, part Environment and Agriculture.

Results and discussion

Planting area of oilseeds
During 2010-2013, the planting area of oilseeds has increased. The most commonly cultivated oilseed crop in the Czech Republic is winter oilseed rape and its planting area increased every year (Table 1). Other oilseeds such as sunflower, poppy and mustard gradually decreased in the area grown (Table 1).

Table 1. Planting area, production, and yield of oilseeds in the Czech Republic 2010-2013 (CSO, 2013).

Crop	Planting area (1000 ha)				Production (1000 t)			Yield (t/ha)		
Year	**2010**	**2011**	**2012**	**2013**	**2010**	**2011**	**2012**	**2010**	**2011**	**2012**
Oilseed rape	368.82	373.39	401.32	418.81	1,042.42	1,046.07	1,109.14	2.83	2.80	2.76
Sunflower	27.17	28.55	24.53	21.28	57.36	70.90	56.94	2.11	2.48	2.31
Soybean	9.47	7.58	5.74	6.51	16.14	17.93	13.15	1.70	2.36	2.29
Poppy	51.10	31.50	18.36	20.25	23.69	26.92	12.81	0.46	0.85	0.70
Mustard	26.82	18.12	16.95	16.47	15.59	16.83	15.47	0.58	0.93	0.91
Oilseed flax	4.09	2.48	1.68	1.51	3.93	3.43	2.40	0.96	1.39	1.43

Weather description
The weather conditions in the Czech Republic were favorable for cultivation of oilseeds in 2012/2013. Conditions for sowing and establishment were good in autumn. September brought rainfall, which relieved a dry period over summer. At the beginning of October the temperature was above normal. These conditions contributed to the development of fungal diseases in oilseed rape crops. Towards the end of October temperatures fell which brought snow. Snow lasted until spring in some higher parts of the country but in lowlands the snow melted. By January the low temperatures and strong freeze had a noticeable impact. In Europe the arrival of spring was late and weather patterns were perturbed. While Finland and most of northern Europe got record high temperatures, western- and central Europe faced much cooler weather and even their wettest May and June ever. This was very exceptional spring for the Czech Republic. During summer prolonged heat waves set new record high temperatures (CHMI, 2013).

The most important diseases and pests of oilseeds in season 2012/2013
Farmers planted their oilseeds according to the integrated plant protection rules and used appropriate pesticide treatments to reduce losses from pests and diseases. The autumn period was favorable for fungal diseases due to warm, wet weather conditions. On oilseed rape symptoms of light leaf spot and *Alternaria* leaf spot were observed from mid- September and

in the middle of October first symptoms of *Phoma* leaf spot appeared. Clubroot and downy mildew occurred, but were localized and did not cause severe damage.

The cold spring and hot summer did not support the development of fungal diseases on spring-sown oilseed rape. Symptoms of *Phoma* leave spot were observed during spring, and in late July, symptoms of white rot were observed. However, no significant damage was recorded. Pest incidence was important. Slugs, coleseed sawfly (*Athalia rosae*) and cabbage root fly (*Delia radicum*) occurred on oilseed rape. In spring, medium level infestation of stem weevils occurred. In Moravia predominated *Ceutorhynchus pallidactylus* and in Bohemia *Ceuthorhynchus napi*. In this season there were problems with pollen beetle on oilseed rape, but most of farmers prevented significant damage by using insecticides with different mode of action according to an anti-resistance strategy. Spring oilseeds were sown with a delay, the possibility to enter the field was late due to the long, cold spring.

On sunflower fields white rot and gray mold were observed, as usual, but these diseases did not cause such damage as in previous years. The main pests were aphids (*Brachycaudus helichrysi*, *Aphis fabae* and others) as usual.

The evidence of the healthy state of the poppy crop was an abnormally high yield. In some regions, farmers reached about 2 t/ha. Although some fungal diseases occurred (mostly downey mildew, leaf blight and gray mold) their incidence was not severe. In southern parts of the Czech Republic, where the climate is warmer, pests such as the poppy root weevil, poppy capsule weevil and stem sawfly occurred at higher incidence levels than in northern parts of the country.

In spring, the young plants of mustard were severely attacked by coleseed sawfly. The most important disease was white rot caused by *Sclerotinia sclerotiorum*. A higher incidence of sclerotia of the pathogen was recorded in seeds compared with the seed quality in 2012.

The biggest problem of flax (oilseed and fiber flax) from the perspective of pests was the incidence of flea beetle (*Aphtona euphorbiae* and *Longitarsus parvulus*). The second important pests of oilseed flax were thrips. Thrips damage occurs more in years with a dry and warm spring, but in 2013 an unusual number of damaged plants were found.

Table 2. The most frequent diseases and pests of oilseeds in the Czech Republic in 2013.

Crop	**Oilseed rape**	**Sunflower**	**Poppy**	**Mustard**	**Oilseed flax**
Disease	Blackleg White rot Clubroot Light leaf spot	White rot Gray mold	Downey mildew Leaf blight Gray mold	White rot *Alternaria* spot	Powdery mildew Pasmo (*Septoria linicola*)
Pest insect	Coleseed sawfly Cabbage root fly Stem weevils Pollen beetle Brassica pod midge Cabbage aphid	Bean aphid Leaf curling Plum aphid	Poppy root weevil Poppy capsule weevil Stem sawfly Bean aphid	Coleseed sawfly Flea beetle	Flea beetle Thrips

The most important diseases and pest in the Czech Republic in season 2012/2013 are shown in Table 2. In general, the exceptional course of weather conditions in Central Europe complicated the crop management and affected the growth and development of oilseed crops, but finally in the vast majority of regions, farmers reached abnormally high yields, especially in winter oilseed rape and poppy.

Acknowledgement

Presented results were obtained from project support NAZV QJ131022.

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IOBC-WPRS Bulletin Vol. 104, 2014
pp. 17-18

Spatial distribution of root maggot larvae (*Delia radicum*) and club-root symptomatic plants (*Plasmodiophora brassicae*) in winter oilseed rape

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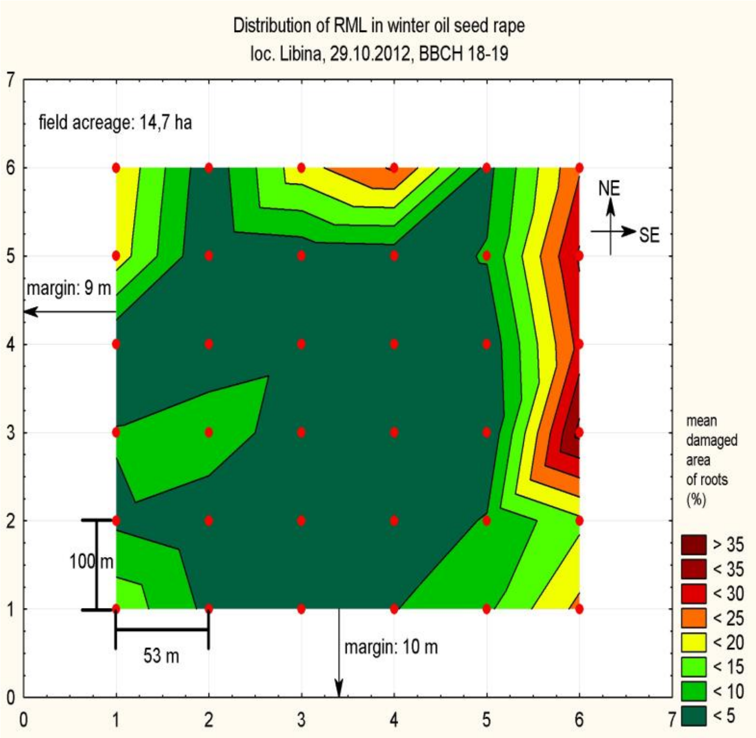
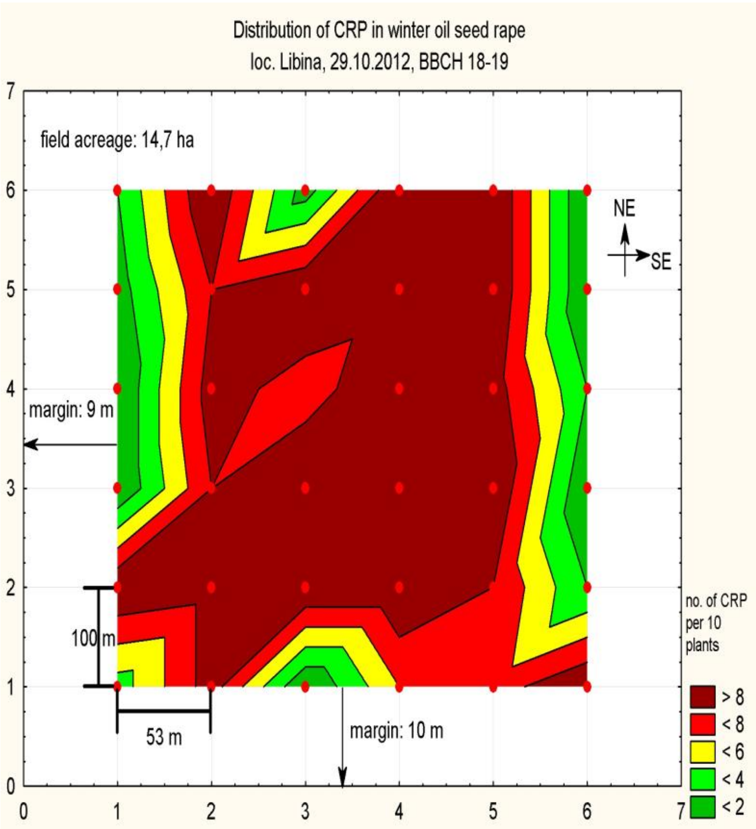
Abstract: Damage caused by root maggots (*Delia radicum*) and club root (*Plasmodiophora brassicae*) in winter oilseed rape fields in the Czech Republic (CZ) have been increasing recently. The distribution of root maggot larvae (RML) and club-root symptomatic plants (CRP) in winter oilseed rape were assessed on six fields within the Olomouc region in CZ during autumn 2012 (Spatial Analysis by Distance IndecEs; SadieShell 1.22). On all localities the plants at the field edges closest to the field margin were more infested by RML (Figure 1). However, it would be incorrect to conclude that the insect pest threatens only field margins. The distributions of RML in fields were not uniform; individuals mostly aggregated. The size and orientation of the infestation focus (places with markedly higher levels of root infestation) were different in the fields we compared. They did not occur only along field margins. Significant positive correlations between the damaged area of roots and the width of hypocotyls were recorded on four localities (Spearman´s **r**: 0.38 - 0.59; $p < 0.05$). Significant positive correlation between the portion of plants infested by RML and the width of hypocotyls occurred only at two localities (Spearman´s **r**: 0.41 - 0.47; $p < 0.05$). CRP were recorded from four localities. Three localities were slightly infested; CRP were concentrated in one or two focus points. One locality (Libina) was severely infested and an almost uniform distribution of the CRP was recorded here (Figure 2). At this location, a significantly negative correlation between the proportion of CRP and the damaged area of roots (caused by RML) was found (Spearman´s **r**: - 0.56; $p < 0.05$), (comparison of Figures 1 and 2).

Key words: *Delia radicum*, clubroot, oilseed rape, spatial distribution

Acknowledgement

This work was funded by the grant QJ1230077 from the Ministry of Agriculture of the Czech Republic.





Figures 1, 2. Distribution of root maggot larvae (above) and club-root (below) symptomatic plants in winter oilseed rape in Libina, Czech Republic (12.10.2012)

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Effects of different soil cultivation after oilseed rape on the increase of *Plasmodiophora brassica* and *Delia radicum*

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Abstract: In terms of the increasing density of cultivation of winter oilseed rape and the increasing trend to modern cost-efficient management practices, the oilseed rape stubbles remain on the soil surface more often. Therefore the volunteer oilseed rape grows for a longer period on the harvested areas. The presence of volunteer oilseed rape can lead to the proliferation of different pathogens and pests in oilseed rape fields. In particular, the third generation of *Delia radicum* develops on the oilseed rape stubble and clubroot disease occurs on the volunteer oilseed rape plants. In this project the effect of soil cultivation after rapeseed harvest on the pathogen and pest was investigated.

Field experiments were done for three years at Julius Kühn Institute in Braunschweig. Different soil cultivations (plow, cultivator and disc harrow) were applied at two different time points (2 weeks after harvest or 4 weeks after harvest). The field trials were observed separately for the occurrence of clubroot and *Delia radicum*.

The results show that the development of clubroot disease can be reduced by a flat tillage at least two weeks after harvest. Later treatment led to a strong increase of clubroot disease. A total reduction of clubroot with different tillage systems is not possible. The observed result on *Delia radicum* was similar to clubroot disease. A reduction of the rate of flies was reached in all treatments. The best results (70% reduction) were achieved by the plow and the cultivator.

Key words: club root cabbage root fly, tillage, phytosanitary effect

Entomology papers

Brassica pod midge
(*Dasineura brassicae*)

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IOBC-WPRS Bulletin Vol. 104, 2014
pp. 25-28

The expanding oilseed rape insect pest community in Estonia

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Abstract: The increase in oilseed rape cultivation over the last two decades has led to the introduction of a “new” pest to Estonian farmers. The brassica pod midge, *Dasineura brassicae* (Winnertz, Diptera: Cecidomyiidae) is a pest of potential economic importance in Europe. Its presence in oilseed rape fields in Estonia became clear during a study conducted to measure the number of overwintered oilseed rape pests and hymenopteran parasitoids within fields and in field edges. Soil samples were collected and incubated in emergence traps from different fields that grew winter oilseed rape in the previous year in Tartu County. Only a low number of pests emerged from these samples from which we can assume that despite the widespread opinion amongst Estonian farmers, most pests do not stay in or near the fields for overwintering. However, the most abundant pest collected was the brassica pod midge, indicating that it does prefer to stay in or near the fields for winter. Additionally, significantly more parasitoids emerged than pests indicating that these natural enemies also chose their overwintering sites within or near the fields. Hence, minimal tillage and a well-planned, diverse crop rotation of at least five years should be favoured to lower the threat of this relatively new pest.

Key words: *Dasineura brassicae*, *Meligethes*, *Ceutorhynchus*, parasitoids, oilseed rape pests

Introduction

Oilseed rape is the most profitable oil crop in the Baltic countries. Its intensive cultivation in Estonia started in the early 1990s on 1.2 thousand hectares; since then the growth area has been increasing, reaching a peak in 2010 (98.2 thousand ha) (Statistics Estonia, 2013). This rapid increase has led to the population growth of specialist pests and might have been one of the factors that triggered the appearance of a new pest on oilseed rape.

There are two key insect pest species attacking oilseed rape in Estonia, the pollen beetle (*Meligethes aeneus* Fab.) mainly on spring oilseed rape and the cabbage seed weevil (*Ceutorhynchus obstrictus* Marsh.) mostly on winter oilseed rape (Veromann *et al*., 2006a,b). The biology of these pests and their natural enemies (hymenopteran parasitoids) has been studied by the researchers of the Estonian University of Life Sciences since the beginning of the 2000s. In 2011, a new pest, the brassica pod midge (*Dasineura brassicae* Winn.) has appeared in oilseed rape fields. This multivoltine pest infests both spring and winter oilseed rape (Williams *et al*., 1987a,b), using mostly the feeding and egg-laying holes made by the cabbage seed weevil to oviposit into pods, amplifying its otherwise little damage to the crop.

The aim of our study was to determine whether the brassica pod midge has established its population and can they overwinter under Estonian climate conditions. For that purpose we measured the number and species composition of overwintered insect pests and hymenopteran parasitoids within winter oilseed rape fields and on field edges.

Material and methods

Soil samples were collected from four fields in Tartu County on the 12th of April 2012. All these fields grew winter oilseed rape in the previous year and were yet without any tillage by the time of sampling. The average daily temperature stayed below 6 °C by the sampling time (-0.6 °C in February, 0.2 °C in March and 5.3 °C in April) (EMHI, 2013). Therefore, oilseed rape related pests did not yet start emerging from their hibernation sites.

Samples were taken from 0, 3 and 25 meters into the field from the field edge, and from 3 meters outside the field in three replicates. There were 12 samples from each field, 48 samples in total. From each sampling point the upper 10 cm from a 25 x 25 cm area was collected into plastic bags, and then transported to the laboratory.

The samples were placed into emergence cardboard boxes and were incubated there until the 25th of June. All emerged insects were counted, sorted and identified.

The data were analysed using STATISTICA 9.1 (StatSoft, Inc). To compare the number of emerged pests and parasitoids Sign test was carried out. The dependence of insect abundance on species and the effect of distance from the field edge on different insects were analysed using Kruskal-Wallis ANOVA test. Differences between varieties were detected with Duncan's post hoc test.

Results and discussion

During the three months of this study 553 hymenopteran parasitoids and 135 oilseed rape pests emerged from our samples (Figure 1). Significantly more beneficial insects than pests were reared ($Z = 4.37$, $n = 41$, $p < 0.0001$). Most of these parasitoids belonged to the Chalcidoidea superfamily (334 specimens).

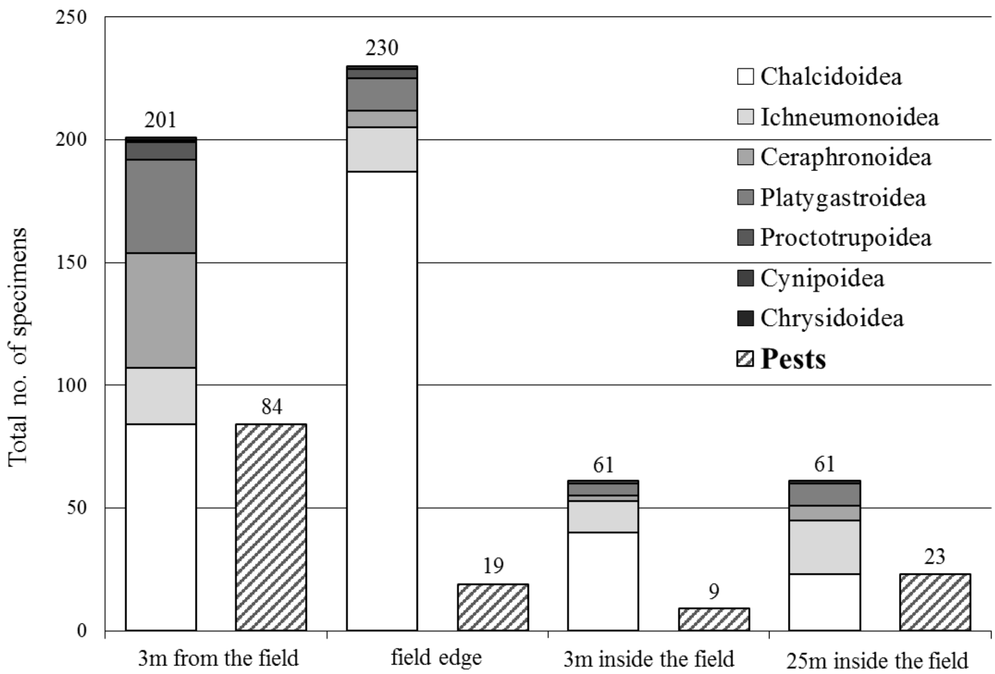

Figure 1. Total number of parasitoid superfamilies and pests emerged from samples collected from and near oilseed rape fields, from different distances.

The most abundant pest found was the brassica pod midge (92 specimens) confirming its presence in oilseed rape fields of Estonia and that it does overwinter in the top 2-5 cm of soil in those fields that grew oilseed rape in the previous year. There were only a few pollen beetle and weevil specimens found. This finding is congruent with previous studies, indicating that these pests search for overwintering sites elsewhere. According to Alford *et al*. (2003) pollen beetle adults chose to spend the winter under the litter layer in non-crop areas, e.g. woodlands as well as grasslands (Rusch *et al*., 2012). Their abundance in overwintering habitats is influenced by the characteristics of those local habitats and the adjacency of oilseed rape crop fields (Rusch *et al*., 2012).

Flea beetles were represented with 38 specimens and their abundance depended on the distance from the field edge (Kruskal-Wallis ANOVA $H_{(3;48)} = 15.96$, $p = 0.0012$). Most of them emerged from samples taken from 3 meters outside the fields, significantly more than from those taken from the field edge ($p = 0.013$) or from inside the field (3 m: $p = 0.0033$; 25 m: $p = 0.0039$).

Insect abundance depended on their species (Kruskal-Wallis ANOVA $H_{(3;189)} = 31.07$ $p < 0.0001$). There were significantly more pod midge adults than pollen beetles ($p = 0.0079$) and ceutorhyncid weevils ($p = 0.0071$); there were no other differences among species (Figure 2).

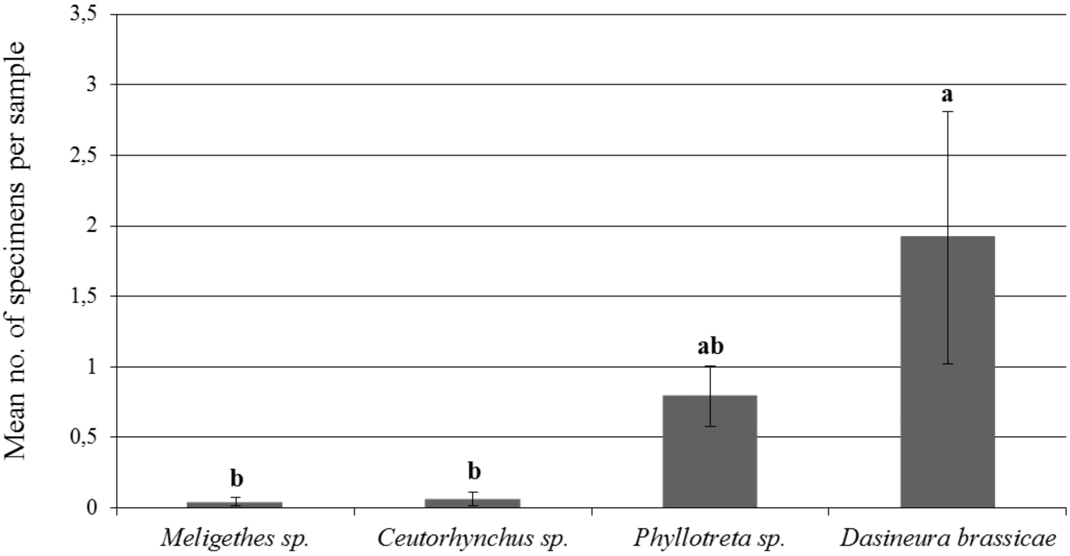

Figure 2. Mean (±SE) number of pests emerged per sample in 2012, Tartu County. Different letters indicate significant differences ($p < 0.05$).

Distance from the field edge did also affect the abundance of beneficial insects (Kruskal-Wallis ANOVA $H_{(3;48)} = 11.39$, $p = 0.0098$). More hymenopteran parasitoids emerged from samples collected from the field edge and 3 meters outside from the field. There were significantly more parasitoids emerging from field edge samples than from inside of the field (3 m: $p = 0.029$; 25 m: $p = 0.031$); highlighting the importance of field edges in the conservation of beneficial insects.

To conclude, the brassica pod midge is indeed present in Estonian oilseed rape fields and prefers to stay in or near the fields for the winter. Further studies are needed to assess the significance of the damage made by this pest, until then a well-planned and diverse crop rotation is an important preventive method to control its abundance. However, this might become a problem as the area for growing oilseed rape is increasing. This study also indicates that the main enemies of Estonian oilseed rape growers do not stay in the soil of the fields for the winter, but search for suitable overwintering sites elsewhere. Natural enemies of these pests however do stay in or near the fields, hence minimum cultivation after oilseed rape may enhance parasitoid survival. Our results support the fact that field edges are important semi-natural habitats in the agricultural landscapes for preserving natural enemies.

Acknowledgements

This study was supported by the Estonian Foundation grant 8895, the projects SF0170057s09 and P9003PKPK of the Estonian Ministry of Education and Research and the European Social Fund's Doctoral Studies and Internationalisation Programme DoRa, which is carried out by Foundation Archimedes.

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Cabbage stem flea beetles (*Psylliodes chrysocephala*)

Integrated Control in Oilseed Crops
IOBC-WPRS Bulletin Vol. 104, 2014
pp. 31-37

Early plant injury as an indicator of infestation level of the cabbage stem flea beetle?

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Abstract: Forecasting abundance of the cabbage stem flea beetle, *Psylliodes chrysocephala,* is widely based on monitoring of adult beetles with yellow water traps but there is not always a direct correlation between trap catches and larval density. The objective of this study was to investigate assessment of early plant injury as a monitoring method. This was done in a field cage experiment, by testing the relationship between beetle density and immediate and subsequent plant injury from adults feeding on plants and from larvae mining plant stems. Overall feeding of adults was low. A statistically significant relationship between beetle density and plant injury was not found when plant injury was expressed as damaged plants nor feeding holes. However the experiment revealed a small but significant increase in the number of damaged leaves: 3.3% per beetle at increasing density of beetles. The observed mean number of damaged leaves per cage of 24 plants was 11.6, 13.8, 13.2 and 19.0 at 2, 4, 8 and 16 beetles, respectively, four weeks after the beetles were released into the cages at plant growth stage BBCH 18. There was a statistically significant relationship between beetle density and larval density per plant. The larval density was low and the observed mean numbers of larvae per plant were 0.15, 0.38, 0.87 and 1.42 at 2, 4, 8 and 16 beetles. Overall the present study demonstrates a correlation between adult beetle density and subsequent number of larvae per plant. However, early plant injury was not a satisfactory indicator of infestation level. The possible causes of low levels of feeding and larval density per plant are discussed.

Key words: Monitoring, forecasting, Cabbage stem flea beetle, *Psylliodes chrysocephala*, plant injury, larval density

Introduction

The cabbage stem flea beetle, *Psylliodes chrysocephala*, is regarded as one of the most important pests in winter oilseed rape, causing damage at time of establishment in autumn and throughout winter. The initial damage is caused by the adults feeding on leaves of the emerging crop. Subsequent damage can be severe and is caused by larvae tunnelling and feeding within leaf petioles and stems, occurring from autumn until the following spring. Larval damage can increase the risk of plant death during winter and can lead to stunted growth in spring (Nilsson, 2002).

Forecasting is widely based on monitoring of the adult beetles with yellow water traps at the time of field invasion (Hossfeld, 1993; Nielsen, 1994; Johnen & Meier, 2000). This forecasting incurs some major limitations since monitoring occurs during a non-damaging stage of the pest and many biological events take place from the time of monitoring until larval damage is visible; i.e., oviposition, hatching and larval development. Embedded in the existing strategy is therefore the uncertainty of whether or not a correlation exists between trap catches and subsequent larval density. A simple correlation does not always exist (Hossfeld, 1993; Johnen & Meier, 2000).

After immigration into the crop, the adult beetles feed on leaves making characteristic damage “shot holes”. A control threshold of 10% damaged leaf area until growth stage BBCH 14 occurs and is used in Germany and Denmark for targeted control of adult beetles (Nielsen G.C., The Knowledge Centre for Agriculture, pers. com.). This control threshold is aimed at protecting the young crop from damaging levels of feeding and does not relate plant injury to larval density or the major damage they cause.

Feeding is affected by temperature, but can be assumed to be more or less equal among beetles under the same conditions (Schulz, 1985). If this assumption holds, then monitoring of feeding can potentially be an indicator of beetle density on a rough scale. Assessment of plant injury can be categorised into incidence and severity of damage. Incidence is the number or percentage of either damaged plants or leaves or number of shot holes. Severity is the percentage of damaged leaf area. The latter is difficult to assess objectively. EPPO (2002) guidelines recommend ‘assessment of damage incidence’ (EPPO, 2002). Monitoring of damage incidence can be done on several levels; by binomial sampling where one plant or one leaf is assessed as ‘with or without visual damage’ and by monitoring the number of shot holes per plant. Which level of assessment will depend on the feeding behaviour of the cabbage stem flea beetle; whether they stay and feed on one plant or move around between plants and leaves.

A field cage experiment was carried out as a first step to developing an alternative monitoring method based on assessment of initial plant injury from adults feeding on plant. The relationship between beetle density and initial plant injury was tested at three levels: the number of plants with damage, the number of leaves with damage and the number of shot holes. The relationship between adult beetle density and subsequent larval density per plant was also tested as was the relationship between initial plant injury and subsequent larval density.

Material and methods

Experiments

A field cage experiment in which different numbers of adult beetles were released into twenty cages containing winter oilseed rape plants was carried out in 2012 in Taastrup, at the experimental farm of Copenhagen University, Faculty of Sciences, Denmark. The experiment aimed to represent low to high infestation levels and included four levels of pairs of beetles (1, 2, 4 and 8) with five replicates per treatment.

The crop was sown on August 20th 2012. A hybrid cultivar (‘DK Expower’), seed-treated with Cruiser (active ingredients Thiamethoxam, Fludioxonil and metalaxyl-M) was used. Row width was 12 cm and plant density was aimed at 40 plants/m^2. Weeds were controlled with Command (active ingredient Clomazon) on August 23rd.

Cages were modified hotbeds (120 x 80 cm with height between 30-38 cm). Cages were placed in the field on September 7th at growth stage BBCH 12-13 in positions of even germination in the field, with a distance of at least 5 m between each cage. Plant density was assessed on September 11th and plants removed to reach a density of 24 plants per cage corresponding to 40 plants/m^2. The outer 10 cm inside the cages was kept free of plants giving a crop plant area of 100 x 60 cm.

The beetles used for the experiment were collected at harvest of winter oilseed rape at the end of July 2012 at time of their summer diapause. They were stored in incubators at 16 °C and a photoperiod of 12:12 h in big plastic containers with stalks of oilseed rape pods. The sex of beetles was determined based on tarsal morphology (Kaufmann, 1941). Pairs of beetles (male & female) were then placed in small containers from mid-August and fed Chinese cabbage until release into the cages on September 11th at BBCH 12-13. The first egg-laying of beetles was observed before release on September 3rd.

Data on the mean daily temperature and precipitation was obtained from the nearest weather station (Danish Meteorological Institute, 2013) and was normal for the period of the experiment according historic records (1961-1999).

Plant injury assessment

Plant injury was assessed in cages once or twice weekly from September 14th - October 9th. The number of plants and leaves with damage and total number of feeding holes per cage was recorded.

Larval density assessment

The number of larvae per plant was assessed once by destructive sampling of twelve plants from each cage in early winter. Plants were stored at 5 °C until assessment. Each leaf stalk was carefully detached, dissected and the number of larvae counted.

Statistical analysis and models

The combined effect of beetle density and time on plant injury as the number of damaged plants, the number of damaged leaves and the number of feeding holes and on larval density were modelled by Poisson regression with a random effect of cage and analyzed by the GLMER procedure in R. Tests for significant effects and differences were assessed by model reduction and based on Likelihood ratio tests. Mean values of observed plant injury are presented and model estimates of the beetle variability from regression analysis. Observed mean values of larval density are presented with 95% confidence intervals estimated by parametric bootstrapping.

All p-values were evaluated at a 5% significance level. All analyses were carried out in R version 2.14.2 (www.r-project.org).

Results

Plant injury

As expected there was a significant effect of time on plant injury ($p < 0.001$) but there was not a significant effect of the number of beetles on plant injury expressed as the number of plants with damage ($p = 0.34$) or as the number of feeding holes ($p = 0.68$). There was a significant but small effect of the number of beetles on the number of leaves with damage ($p = 0.036$). Feeding was low and the difference in number of leaves with damage at the different beetle densities became more pronounced after 21 days (Table 1). The difference in feeding between 4 and 8 pairs of beetles was negligible. The model estimate showed an increase of mean leaves damaged per cage of 3.3% (± 1.6%) per beetle at increasing beetle density. There was no direct correlation between early plant injury and larvae density per plant.

Larval density

There was a significant effect of the number of beetles per cage on larval density per plant ($p = 0.023$). The majority of larvae found were first instars and larval density per plant was low. The standard deviation of the mean and the estimated 95% confidence intervals increased with increasing number of beetles (Table 2 and Figure 1).

Table 1. Mean number of damaged leaves (±standard deviation) per cage at different intervals (days) after adult cabbage stem flea beetles (pairs) were released into cages.

	Mean number (± sd) of damaged leaves (per cage = 24 plants)					
Beetles	**3 days**	**7 days**	**10 days**	**17 days**	**21 days**	**28 days**
2	1.00 (±1.41)	3.40 (±2.61)	3.20 (±1.92)	5.40 (±2.51)	6.60 (±2.51)	11.60 (±3.36)
4	0.40 (±0.89)	1.40 (±1.14)	1.20 (±1.10)	4.20 (±2.39)	9.80 (±4.97)	13.80 (±5.36)
8	1.20 (±1.79)	3.20 (±3.27)	5.60 (±3.78)	5.20 (±1.92)	9.20 (±4.66)	13.20 (±6.61)
16	1.60 (±1.95)	3.80 (±1.64)	4.20 (±2.39)	6.20 (±2.95)	13.00 (±4.42)	19.00 (±7.00)

Table 2. Mean number of cabbage stem flea beetle larvae per oilseed rape plant (± standard deviation) and estimated 95% CI. CI estimated from model by parametric bootstrapping.

Beetles	Larvae/plant (mean ± sd)	Estimated 95% CI
2	0.15 (± 0.66)	0.033-0.283
4	0.38 (± 0.99)	0.133-0.633
8	0.87 (± 1.59)	0.383-1.633
16	1.42 (± 1.90)	0.817-3.050

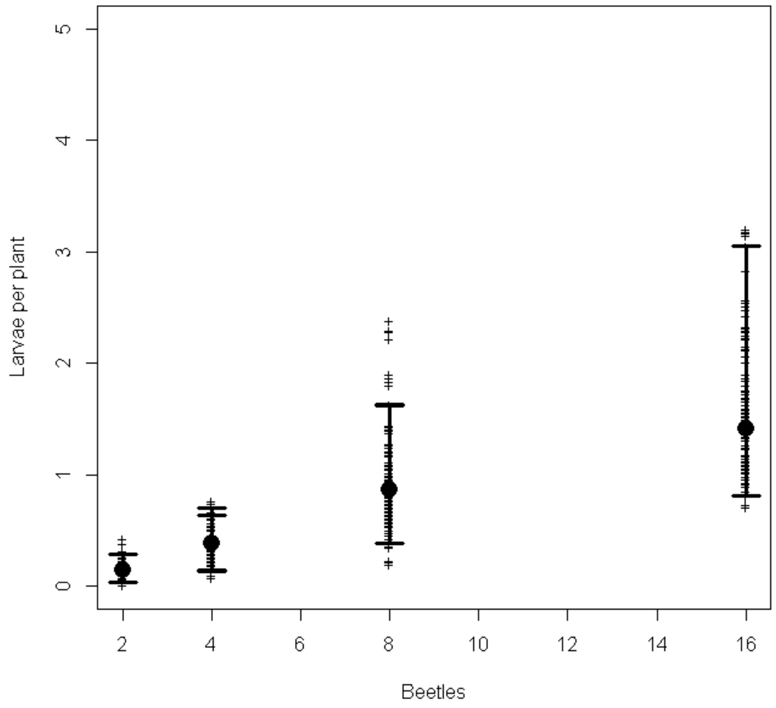

Figure 1. Observed mean number (•) of cabbage stem flea beetle larvae per oilseed rape plant and model estimates of 95% CI at 2, 4, 8 and 16 beetles per cage.

Discussion

In a study by Schulz (1985) a correlation between temperature, feeding and egg-laying was observed with increasing activity and a peak in activity approximately a month after the beetles had started feeding (Schulz, 1985); an observation indicating that feeding affects egg-laying and the potential larval population.

Insect activity in this experiment was low at all levels of beetle densities in terms of feeding, and egg-laying expressed by the subsequent low larval density. Even though plant injury as number of leaves with damage was significantly affected by beetle density, the feeding was low and the increase was only 3.3% more leaves with damage at an increase of one extra beetle per cage. This range of injury levels is not broad enough to distinguish a rough scale between beetle densities and is therefore not useful for practical monitoring and forecasting. The low larval density per plant could be due to low levels of egg-laying, high mortality of eggs and larvae or delayed egg-laying and larval development, so that eggs had not hatched before the time of assessment of larval density.

Weather conditions at time of release were normal according to Danish standards (Danish Meteorological Institute, 2013). Mean daily temperature was 12 °C, a temperature at which egg-laying is characterised as optimal (Bonnemaison and Jourdheuil, 1954; Buehler, 1986). It is often stated that beetles start egg-laying 10-12 days after crop invasion and that the egg-laying peak is around 4 weeks after crop invasion (Bonnemaison & Jourdheuil, 1954; Alford, 1979; Nielsen, 1994; Vig, 2002). Dependant on the onset of egg-laying, the first instar larvae could be present around 200-240 day-degrees (DD°) above 3.2-4 °C (Alford, 1979; Johnen & Meier, 2000). According to earlier findings, the first larvae in this experiment could then be present from around October 22nd based on the mean daily temperatures of the period. The sum of DD° did not permit hatching of eggs laid one month after the start of the experiment, a time corresponding to a peak of egg-laying. Larval development would not have reached the second instar stage. This corresponds well with the finding of this experiment of low larval density per plant and majority of the larvae found being mostly first instars.

Despite this it is noteworthy that the mean larval density at the high density of beetles was as low as 1.42 larvae per plant. Females at the highest density therefore, gave rise to an average total number of 4.26 larvae or less than 0.2 larvae per plant. Five larvae per plant is a former control threshold from Britain (Purvis, 1986). To reach this larval density per plant would then require 25 females per cage or approximately 1 female per plant. In this perspective the results reflect that a density of 16 beetles per cage of 24 plants did not correspond to a high infestation level.

The beetles used may have affected activity. Neither the developmental state of the beetles nor whether summer diapause had ended at time of release into the cages is known. The beetles were collected during their summer diapause and were kept at a constant mean daily temperature of 16 °C for approximately 42 days until the time of release. Earlier studies found diapause to be between 48-62 days under different conditions of temperature, relative humidity and food quality (Bonnemaison & Jourdheuil, 1954; Saringer, 1984). Furthermore, the start of crop invasion or resumed beetle activity has been shown to correspond to a decrease in temperature followed by an increase (Pouzet & Ballanger, 1984). In this experiment the beetles were kept at a constant temperature and then experienced a drop in mean daily temperature from the time of their release.

This first step in evaluating plant injury for improved monitoring did not show plant injury to be a useful and reliable indicator of beetle density. A direct correlation between adult beetle and subsequent larval densities was found, a prerequisite for a monitoring method based on a non-damaging insect stage. If a direct correlation exists under specified weather conditions then monitoring of these conditions can be used to estimate damage risk. These results would benefit from further experiments using a broader range of beetle densities. Furthermore the use of beetles collected during crop invasion would ignore the uncertainties related to summer diapause and female reproductive status.

Acknowledgements

We thank Anders Nørgaard for assistance in the field, Holger Philipsen for assistance in the field, useful comments and reviewing the manuscript and Ghita C. Nielsen for inputs and comments. We thank Anders Tolver, Bo Markussen and Helle Sørensen for statistical advice. The work was funded by Ministry of Science, Innovation and Higher Education and The Knowledge Centre for Agriculture.

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Integrated Control in Oilseed Crops
IOBC-WPRS Bulletin Vol. 104, 2014
pp. 39-40

Challenges and potential for organic rapeseed production in Denmark

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Abstract: The current organic oilseed rape area in Denmark is approximately 470 ha (Danish AgriFish Agency, 2012). However, the Danish market potential for organically grown oilseed rape is at least ten times higher than the current production (Nilsson, A. J., Statistics Denmark, personal communication June 2012). This gap between supply and demand is primarily due to frequent and severe attacks by two insect pest species, the cabbage stem flea beetle [*Psylliodes chrysocephala* (L.)] and the pollen beetle (*Meligethes aeneus* F.).

In autumn and winter, cabbage stem flee beetle adults feed on seedling winter oilseed rape and can cause considerable damage. Their larvae tunnel leaf stalks and stems. The pollen beetle adults feed on pollen. On a flowering crop this is not problematic, however prior to flowering the adults bite holes into the green buds to feed on the pollen. The pistil is often damaged, resulting in sterility or premature loss of the flower.

Often organic rape seed crops have to be ploughed under in spring because of low crop plant survival after attacks by the cabbage stem flea beetle. Even with acceptable crop densities in spring, attacks by invading pollen beetles may subsequently minimize seed setting. Therefore, oilseed rape is considered a high risk crop to grow for Danish organic farmers.

A current Danish project (financed by two national funding bodies: 'Fonden for Økologisk Landbrug' and 'Promilleafgiftsfonden') is investigating management strategies to prevent and/or reduce attacks by insect pests in organically produced oilseed rape in order to increase the organic oil seed rape production. A further goal of this project is to initiate a transnational exchange of knowledge and practical experiences combined with development of pest control strategies e.g. conservation biocontrol, inundation and inoculation biocontrol, and also trap cropping.

Currently, a Danish field experiment with different management tactics are being conducted in commercially grown winter oilseed rape fields at 3 locations. The experimental treatments are based on promising non-chemical control methods, described in the literature, targeting the cabbage stem flea beetle. Five different management tactics have been chosen: mixing oilseed rape with turnip, which has been found more preferable to the cabbage stem flea beetle than oilseed rape; physically covering the crop plants with wood pellets at the early stages; lime stone treatment to create an environment unsuitable for egg laying; physically drying out eggs by repeated hoeing/harrowing; and hoeing to cover the base of the young oilseed rape plants with soil (Barari *et al.*, 2005; Daniel & Dierauer, 2013). Prerequisites for the chosen experimental sites are that the fields are certified as organic, the previous crops were leguminous, and the most recent oilseed rape crops in the fields were grown at least 4 years ago. Each plot has 2 replicates. The treatments are:

1. Variety Exclusiv, sown with a 12 cm row distance between the rows
2. Variety Ladoga with 25 cm row distance and hoeing at the 1-2 leaf-stage
3. Variety Exclusiv at 25 cm row distance and hoeing at the 1-2 leaf-stage

4. Variety Exclusiv in mixture with turnip, at 25 cm row distance between rows of the mixture, hoeing at the 1-2 leaf-stage of oilseed rape.
5. Variety Exclusiv and turnip in alternating rows. 25 cm between rows, hoeing at the 1-2 leaf-stage of oilseed rape.
6. Variety Exclusiv at 25 cm row distance, a cover of wood pellets is placed at the 1-2 leaf stage in a band surrounding the crop row.
7. Variety Exclusiv at 25 cm row distance, a cover of agricultural lime is placed at the 1-2 leaf stage in a band surrounding the crop row.
8. Variety Exclusiv, at 25 cm row distance, repeated hoeing/harrowing strategy starting at the 1-2 leaf stage with succeeding cultivations at 10 day intervals.

Preliminary results from the ongoing field experiment will be presented on the poster; the final re-sults from the experiments will be assessed in the spring of 2014.

Key words: Organic oilseed rape production, management strategies, cabbage stem flea beetle, *Psylliodes chrysocephala*, pollen beetle, *Meligethes aeneus*

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Parasitism of cabbage stem flea beetle in oilseed rape and turnip rape

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Abstract: The cabbage stem flea beetle (*Psylliodes chrysocephala* L.) is one of the most devastating insect pests of winter oilseed rape in northern Europe. While adult feeding rarely causes economic losses severe damage is commonly caused by larval feeding within petioles and shoots of juvenile plants. Control of cabbage stem flea beetle mainly relies on insecticide applications. In addition, previous studies have shown that natural enemies like the endoparasitic larval parasitoid *Tersilochus microgaster* (Hym.; Ichneumonidae) can have substantial impact on pest populations. This study aimed to compare the larval parasitism of cabbage stem flea beetle in winter oilseed rape and three varieties of winter turnip rape.

In two years' field trials winter oilseed rape (*Brassica napus*, cv 'Robust') and winter turnip rape (*Brassica rapa*, cvs 'Largo', 'Malwira' and 'Perko') was grown in a complete randomized plot design, with four replicated plots of each treatment, in the region of Göttingen (Northern Germany). Larvae of cabbage stem flea beetle were collected from plant samples in March 2010 and April 2011, respectively, and stored in 70% ethanol. The level of parasitism was assessed by dissection of larvae under a microscope. Additionally, subsamples of the collected larvae were reared in the laboratory to adulthood and parasitoid females emerging from parasitized larvae were identified to species level. All parasitoid females were determined as *T. microgaster*.

In both years the level of plant infestation by larvae of cabbage stem flea beetle was higher on turnip rape compared to oilseed rape. This difference was significant only in 2010. There was no significant difference between larval infestation in the three turnip rape varieties. The dissection of larvae of cabbage stem flea beetle resulted in parasitism rates exceeding 50% in some treatments. In 2010, the level of parasitism was significantly higher in larvae collected from the three varieties of turnip rape (ca 50%) than in larvae collected from oilseed rape (ca 23%). In 2011, the parasitism of larvae was on a similar level on oilssed rape and on two varieties of turnip rape, but was significantly lower on turnip rape cv. Malwira. Thus, differences between parasitism rates in the tested species and varieties of host plants were not consistent between years.

Our results confirm the importance of natural biocontrol for regulation of pest populations in crops of winter oilseed rape. The effect of plant species and variety on parasitism of cabbage stem flea beetle differed between years, and might have been affected by annual plant phenology and morphology. Larval parasitism was not significantly correlated with host density.

Key words: *Psylliodes chrysocephala*, larval parasitism, *Tersilochus microgatser, Brassica napus, Brassica rapa*

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Field monitoring of cabbage stem flea beetle and rape winter stem weevil autumn flights

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Abstract: Cabbage stem flea beetle (*Psylliodes chrysocephala*) and rape winter stem weevil (*Ceutorhynchus picitarsis*) are two important pests of winter oilseed rape in France. Adults of these two species colonize fields in autumn. Weevil adults are not harmful unlike flea beetle adults that can cause important damage when they arrive on very young crop plants. However, for both species, the larvae are more harmful. They grow in leaf stalks in winter and autumn and can migrate into the stem and destroy the terminal raceme.

Since 2009/2010, infestations by these two species have become very important in some French areas and despite repeated treatments, farmers are unable to control them. Several hypotheses can explain this situation including reinfestation, and resistant populations to pyrethroids. Since autumn 2011, CETIOM (The Technical Center for Oilseed Crops and Industrial Hemp) began studies to understand the phenomenon. This study has two main purposes: (i) to determine if colonisation happens through several flights which could explain why treatments are ineffective; (ii) to acquire biological data to determine biological traits like the stimuli inducing flights or the delay before egg laying for weevils, which could help farmers to spray at the right time.

In France, flea beetles usually colonize fields via one massive flight induced by a fall followed by a climb of maximal temperatures around 20 °C. The monitoring revealed some exceptions: several flights were observed with unknown causes. Unlike the flea beetle, rape winter stem weevils colonize fields via several flights. The monitoring confirms that catches of *C. picitarsis* in yellow traps are not representative of the actual infestation in fields, and shows the limit of this tool to manage this pest. We also checked the delay before egg laying, because in France farmers are recommended to spray just before the observation of the first egg in plants since pyrethroids are not effective against eggs or larvae. This delay is historically estimated around 8 to 10 days after field colonisation. We noticed that this delay could be longer when flights arrive early in autumn.

Key words: Cabbage stem flea beetle, *Psylliodes chrysocephala*, rape winter stem weevil, *Ceutorhynchus picitarsis*

Stem weevils
(*Ceutorhynchus pallidactylus & C. napi*)

Population dynamics and sex ratio of adult forms of stem mining weevils in Croatia

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Abstract: In oilseed rape two stem weevil species occur, the cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsham, 1802) and the rape stem weevil (*C. napi* Gyllenhal, 1837). Both are among the most important oilseed rape pests that appear in spring. The monitoring of appearance of stem mining weevils was conducted in period from 2010 to 2012. Four yellow water traps were set up in the field at the beginning of leaf development (BBCH 12). Samples were collected once a week until harvest. According to morphological marks on the legs, the sexes were determined. The first peak of the flight of cabbage stem weevil usually occurs in February and both sexes immigrate at the same time but in different proportions. Males and females of the rape stem weevil start to immigrate two to three weeks later in the spring and in lower abundance compared to the cabbage stem weevil. As a consequence of the different time of appearance and sex ratio, two insecticide applications are necessary for their successful control.

Key words: Oilseed rape, stem mining weevils, population dynamic, sex ratio

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Effects of various resyntheses, lines, and cultivars of oilseed rape on rape stem weevil (*Ceutorhynchus napi* Gyll.) infestation

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Abstract: In Germany oilseed rape crops are treated each year several times with insecticides. High levels of pest infestation are accompanied by increasing resistance of oilseed rape pests to the registered pyrethroid insecticides. To minimize the number of insecticide applications, resistant cultivars are needed in integrated pest management systems. Genetic host plant resistance might provide a promising alternative to the extensive use of chemical plant protection products. The univoltine rape stem weevil, *Ceutorhynchus napi* Gyll. (Col., Curculionidae), is one of the major pests of winter oilseed rape (*Brassica napus* L.) in Europe. Yields of oilseed rape can be reduced by stem mining weevils by up to 20%.

Nine *Brassica napus* genotypes showing a broad genetic variability were evaluated in a field trial with 4 replicated plots of each genotype in 2012/2013. To assess the oviposition and larval infestation of rape stem weevil, the number of eggs and larvae in the stem pith of genotypes were recorded from plant samples collected at weekly intervals during the infestation period in April and May 2013. Additionally, the length of the stem and the C:N ratio of the stem tissue was measured.

Genotypes differed not only with respect to their stem length but also to the C:N ratio of the stem tissue. We found that the plant genotype significantly influenced plant infestation by rape stem weevil. The resynthesis S30 showed the lowest, and the old (++) cultivar Sollux showed the highest infestation level. The number of eggs and larvae per plant significantly differed between the genotypes on several sampling occasions. Different ratios of the number of eggs laid and number of larvae developing within individual genotypes indicated antibiosis effects. We also found a negative correlation between stem length on April 22nd and the number of eggs per plant on April 29th. Furthermore, negative correlations were determined between the total number of eggs and larvae and the stem length on several sampling occasions. Although the C:N ratio is known to affect performance of herbivorous insects, we found no correlation between the C:N ratio of stem tissue and rape stem weevil infestation.

We conclude that stem length has an influence on oviposition performance of *C. napi* and that other plant factors affect egg and larval development during the infestation period. More investigations are needed to identify possible other traits responsible for pest resistance, for instance secondary metabolites like glucosinolates.

Key words: Insect resistance, plant breeding, oilseed rape, *Ceutorhynchus napi*, oviposition, larval abundance, stem length

Cabbage root fly (*Delia radicum*)

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Screening of *Brassica napus*, *Sinapis alba* and intergeneric hybrids for resistance to cabbage root fly (*Delia radicum* L.)

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Abstract: The cabbage root fly (*Delia radicum* L.) (Dipt.; Anthomyiidae) is of increasing importance as an insect pest in oilseed rape production in Europe. Plant damage occurs when larvae feed on the root tissue of young plants. In recent years the larvae of this pest have been controlled by neonicotinoid insecticide seed coating. The usage of this group of insecticides as seed treatment will be strongly restricted in the EU from December 2013 onwards. Hence, there is an urgent need to find alternative strategies for control of cabbage root fly. Cultivars of oilseed rape resistant to the cabbage maggot could comprise an important component in an IPM system.

No-choice feeding experiments were conducted under controlled environmental conditions (19 ± 1 °C; 18 h L : 6 h D photoperiod) to evaluate the resistance levels of 30 *Brassica* genotypes (*S. alba*, *B. napus* and introgressions of *S. alba* x *B. napus*) to the cabbage maggot. Potted test plants were artificially infested with eight eggs/plant at growth stage BBCH 15-16. Upon hatching the larvae were allowed to feed on plants for a four week time span. At the end of the experimental period the roots were scored for the extent of damage and the soil around the roots was examined for larvae and pupae. Individual larvae or pupae were counted and weighed. The taproots were washed and the degree of root injury was evaluated.

Among the genotypes tested only the cultivars of *S. alba* showed a significant reduction of feeding damage and survival of the larvae. There were only minor differences between root damage and larval performance on the tested intergeneric hybrids and the *B. napus* cultivars. Thus, further introgressions are required to transfer the resistance mechanisms of *S. alba* into genotypes of *B. napus*.

Key words: Insect resistance, plant breeding, oilseed rape, *Ceutorhynchus napi*, oviposition, larval abundance, stem length

Parasitism and effects of insecticides

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A study to assess the parasitism of insect pests in winter oilseed rape in Belgium: preliminary results

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Abstract: A survey of the parasitoids found in commercial winter oilseed rape was initiated in 2012 and 2013 in the South part of Belgium, using both aerial sampling techniques and soil analysis. Fourteen fields located in two distinct areas and with two different tillage regime (normal and reduced or no tillage) were selected for before and just after flowering. Adult parasitoid hymenoptera were weekly sampled over 8 weeks using sweep net. Pollen beetle larvae, *Meligethes aeneus* (F.) (Col.; Nitidulidae), were collected and their parasitism rate assessed. Samples of soil were taken from 4 fields in 2012 and 8 in 2013 to collect brassica pod midge cocoon, *Dasineura brassicae* (Winnertz) (Dip.; Cecidomyiidae), and to assess their parasitism. The soils were gently washed into sieves and cocoons were isolated in Petri dishes until midge or parasitoid emergence.

The main parasitoid wasps found in the sweep net samples belong to the Tersilochinae family. However, though adults of this family were regularly collected in large numbers, and were synchronized with their host, the parasitism level of the pollen beetle larvae remained low, with many of the fields below 10-15% parasitism. Preliminary analysis shows that there were no apparent differences between the two distinct areas and between the two different tillage regimes. The main explanation of this low parasitism rate could be the high occurrence of the insecticide applications, as most of the farmers regularly applied one or two insecticides during the season: the first to control pollen beetle before flowering and the second to control other insects later (e.g. seed weevil, brassica pod midge). The highest level of parasitism of pollen beetle larvae (43%) was found in an untreated field. The identification of the species is in progress.

The analysis of brassica pod midge cocoons showed that the parasitism rate was low in 2012 (0-5%). However, these results were probably underestimated due to a high mortality of the cocoons during the rearing process. If the parasitism rates were expressed on the basis of rearing success (brassica pod midge or adult parasitoid emerged), the parasitism rate reached up to 59.6% in one specific site, with 58.8% due to 4 Ceraphronidae species and 48.6% due to one species, *Ceraphron serraticornis* Kieffer. In 2013, the parasitism rate was low (0-3.0%), despite a high success in the cocoon rearing process.

These results have shown that several species of parasitoid Hymenoptera are present in Belgium, causing in some cases high parasitism levels. A better use of these parasitoid wasps in the biological or integrated control of several oilseed rape pests is possible, but there is a need to focus on improving understanding of the factors that could explain the variability of the parasitism between sites and the actions that could promote the activity of these beneficial insects and protect their existing populations.

Key words: Pollen beetle, Brassica pod midge, parasitism, Tersilochinae, Pteromalidae, Ceraphronidae

Introduction

Winter oilseed rape has become an important crop in the Walloon area, in the south part of Belgium. This crop can be severely attacked by a set of insects and the systematic use of 2 or 3 insecticides during the season is the common practice, with at least one application before flowering to control the main insect problem, the pollen beetle *Meligethes aeneus* (Col.; Nitidulidae). However, this insect has developed across Europe resistance against several insecticides (Hansen, 2003; Heimbach *et al.*, 2006, Thieme *et al.*, 2010) and local Belgian populations were not an exception (De Proft, 2008). If new insecticides compounds were developed and registered to replace the old ones, this is only a short-term solution as new resistance could rapidly appear. Furthermore, the systematic use of insecticides against the pollen beetle is suspected to promote other pests as weevils and the brassica pod midge by impacting their natural enemies. Insecticides applied during the flowering period are also at risk for pollination and honeybees. Therefore, other solutions than the classical insecticide protection are searched.

European research projects (BORIS, 1997-2000 and MASTER, 2001-2005) have put in evidence the high importance of several parasitoid hymenoptera in the biological control of oilseed rape pests and their possible uses in the context of IPM and sustainable agriculture. By example, for the pollen beetle, larval parasitism rates of up to 90% have already been observed in Europe and levels higher than 50% are not uncommon (Williams, 2006; Hokkanen, 2006; Buchi, 1991; Krauss and Krompt, 2002).

The parasitoids of insect pests in winter oilseed rape have been poorly studied in Belgium. By example, larval pollen beetle parasitism (levels, species, etc.) are not known. A new research program was recently initiated to try to fill these gaps, in order to adapt the current practice and reduce insecticide dependence. This is a long term study and the researches are planned for several years. The first results of a prospective campaign carried out in spring 2013 are presented here. In addition to this research, results obtained on the parasitism of the brassica pod midge, *Dasineura brassicae* (Dip.; Cecidomyiidae), in the context of a program based on several Cecidomyiidae in several crops, are also included.

Material and methods

Field sites

The parasitoids of the pollen beetle and the other pest were followed in 2013 in 14 commercial fields. Approximately half of the fields were located in an area of intensive agriculture, with a low landscape diversity (“undiversified landscape” – UL) and the second half were located in an area with a more diversified landscape and an higher proportion of woods, pasture and not cultivated area surroundings the fields (“Diversified Landscape” – DL). Several of these fields, in both areas, were managed by farmers using simplified tillage or direct drilling methods (“Reduced”) in at least a part of their fields and the other ones followed a traditional system with a normal tillage (“Normal”). Brassica pod midge parasitoids were sampled in commercial winter wheat fields seeded directly after oilseed rape and with a high brassica pod midge pressure, located in the Walloon area.

Sampling

The adult pollen beetles were monitored weekly from bud stage to the end of the flowering period using beating methods. The beatings were performed by shaking 5 x 10 terminal parts of plants just above a plastic tray. One sample was performed in the field margins, the four other inside the field. The insects that fell on the trays (mainly pollen beetle) were directly identified and counted. When the first larvae of pollen beetle were detected, this method was replaced by “funnel beatings”, that allowed to harvest pollen beetle larvae. 5 x 10 terminal part of plants (stem, flowers and flower bud) randomly selected into each plots were shaken just above a plastic funnel (Ø 30 cm) placed under a plastic bottle (250 ml). The insects that fell on the funnel were rinsed with water (+ commercial soap) to collect them on the plastic bottle that were brought back to the laboratory for counting, identification and determination of the parasitism rates for the pollen beetle larvae.

For parasitoid hymenoptera sampling, reinforced nets (Ø 35 cm) were used, with 2 x 10 go-back moves (sampling of around 2 x 3-4 m²). The sweep nets were emptied under a funnel, identical to those used for the funnel samplings. One sampling was performed in the field margins, the other one inside the field.

For the brassica pod midge parasitoids, soil samples were taken into 4 fields in 2012 and 8 fields in 2013 in Belgium at the end of the winter period. The previous crop of these 12 fields was winter oilseed rape. Soil sample in one field consisted of 20 sub-sample collected using a bulb planter on 10 cm deep. For extraction of *D. brassicae* cocoons, each soil sample was placed in a 5 l bucket filled with water and then kneaded until its full dispersion. Water containing soil was poured on three successive sieves with mesh sizes of 2.8 mm, 1 mm and 300 µm. This operation was repeated until the entire sample was filtered. Only the sieve of 300 µm retains the cocoons. The retained cocoons were collected, identified and counted under a binocular magnifier. Cocoons were isolated on a wet filter in Petri dishes and were kept till midge or parasitoid emergence that occurred approximately from 2 weeks to 2 months later.

Insect identification and interpretation of results

The parasitism of the larvae of pollen beetle harvested was detected under a binocular. The parasitism was confirmed by the dissection for the first samples. The adult of the parasitoid wasps collected with the sweep net were identified at the family or subfamily level. Identification to the species level for the most important families is planned. The brassica pod midge parasitoids (Ceraphronidae) were identified to the species level by Peter N. Buhl (Swedish Museum of Natural History).

Results

Pollen beetle parasitism

The results of the monitoring of the pollen beetle (adults, larvae with their parasitism rate) and the parasitoids (adults of Tersilochinae) are listed in Table 1 for the 14 fields monitored. The adult and larvae of pollen beetle first detection, the sampling of the first adults of Tersilochinae and the detection of first parasitized larvae were more or less similar in all the fields, with sometimes one week delay between fields. Adults of pollen beetle arrived first, followed by the parasitoid wasps that were present in all the fields when the first pollen beetle larvae were found.

Parasitism was detected in all fields but the levels remains low with a mean of 12.2% during all the season and a lot of fields around or below 10.0% (Table 2). There were no differences between the two main parameters used to characterize the fields: the diversity of the landscape surrounding the fields and the tillage regime. If higher parasitism rates for fields located on area with a diversified landscape and for fields cropped by farmers with simplified reduced tillage systems were observed, this was only a general trend and the differences were not significant.

Table 1. Presence of adult of pollen beetle and Tersilochinae and of pollen beetle larvae, parasitized and unparasitized at the different dates in 2013.

			April				May					June	
			3	10	17	24	1	8	15	22	29	5	12
Waulsort	Pollen beetle	Adult											
		Larvae							----		---		
	Tersilochinae	Adult											
Onhaye	Pollen beetle	Adult											
		Larvae									--	---	---
	Tersilochinae	Adult											
Freyr	Pollen beetle	Adult											
		Larvae							--	---	---	----	---
	Tersilochinae	Adult											
Ernage	Pollen beetle	Adult											
		Larvae								-	.	.	-
	Tersilochinae	Adult											
Temploux	Pollen beetle	Adult											
		Larvae								-	--	--	-
	Tersilochinae	Adult											
Balâtre	Pollen beetle	Adult											
		Larvae							.	--	--	-	--
	Tersilochinae	Adult											
Velaine	Pollen beetle	Adult											
		Larvae							-	--	-	-	-
	Tersilochinae	Adult											
Lamalle	Pollen beetle	Adult											
		Larvae							-			.	.
	Tersilochinae	Adult											
Couthuin	Pollen beetle	Adult											
		Larvae							.	--	-	-	
	Tersilochinae	Adult											
Bas-Oha	Pollen beetle	Adult											
		Larvae									--	-	-
	Tersilochinae	Adult											
Wanze	Pollen beetle	Adult											
		Larvae							.	.	--	---	---
	Tersilochinae	Adult											
Landenne	Pollen beetle	Adult											
		Larvae								--	--	-	-
	Tersilochinae	Adult											
Heron 1	Pollen beetle	Adult											
		Larvae										.	.
	Tersilochinae	Adult											
Heron 2	Pollen beetle	Adult											
		Larvae							-	--	-	-	---
	Tersilochinae	Adult											

Pollen beetle larvae/sample	1-10	10-50	50-100	> 100	
Parasitism rate	. < 5%	- 5-10%	-- 10-25%	--- 25-50%	---- > 50%

Table 2. Parasitism percentage of the pollen beetle larvae collected during the season in the different fields and means by area and tillage system.

	Area	Tillage system	Pollen beetle larvae (all sampling dates)		
			Total	Parasitized	% parasitism
Waulsort	DL	Reduced	54	14	25.9%
Onhaye	DL	Reduced	59	14	23.7%
Freyr	DL	Reduced	350	102	29.1%
Ernage	UDL	Normal	149	6	4.0%
Temploux	UDL	Reduced	284	30	10.6%
Balatre 1	DL	Normal	477	52	10.9%
Velaine	DL	Normal	482	36	7.5%
Lamalle	DL	Reduced	200	5	2.5%
Couthuin	UDL	Reduced	300	24	8.0%
Bas-Oha	UDL	Normal	310	36	11.6%
Wanze	UDL	Normal	336	37	11.0%
Heron 1	UDL	Reduced	120	5	4.2%
Landenne	UDL	Reduced	99	9	9.1%
Heron 2	UDL	Normal	245	29	11.8%
means		All 12.1%			
		Reduced 14.1% a		Div. landscape (DL)	16.6% a
		Normal 9.5% a		Undiv. landscape (UDL)	8.8% a

Binomial GLM, likelihood ratio test ($p = 0.05$). Results followed by the same letter are not different.

The occurrence of the different parasitic hymenoptera sampled with the sweep nets in the different fields are listed in Table 3. A total of 951 adult parasitoids were collected. The main families were Tersilochinae (69.1%) and Pteromalidae (23.6%), with a peak population beginning of May for the first family and beginning of June for the second family. These peaks corresponded to the presence in abundance of pollen beetle larvae in May and seed weevil larvae in June. Even if the specimens were not identified to the species levels, it was assumed that most of the Tersilochinae found were linked to the pollen beetle and that most of the Pteromalidae were linked to the seed weevils, that was particularly abundant in 2013.

The results of the rearing of the brassica pod midge cocoons sampled into the soil to determine their parasitism rates are given in Table 4 for the 3 fields where parasitoids were found. In the 9 other fields, brassica pod midges were harvested (mean of 53 cocoons/field, range 19-85) but no parasitoids were found, while adult midges were obtained with a similar rearing success than the fields with parasitoids. The rearing success of the cocoons (adult of brassica pod midge or parasitoid) was low in 2012 (mean of 27.6%, range 8.4-42.4%) but the method was updated in 2013 (sampling later in the season, management of the humidity when reared, etc.) and the rearing success were increased (mean of 62.7%, range of 19.8-93.7%). A species previously not known to attack the brassica pod midge, *Ceraphron bispinosus* (Hym.; Ceraphronidae), was recorded for the first time during this study, with a presence in the three different locations. In one field, Buzet, the parasitism was particularly high (59.7%) with one species, *Ceraphron serraticornis*, responsible of more than 80% of this parasitism rate. This species was however only found in this field.

Table 3. Occurrence of the different family of parasitoid hymenoptera sampled in oilseed rape with sweep net in the 14 fields in 2013.

	1-May	8-May	15-May	22-May	29-May	5-June	12-June	Total	
Tersilochinae	45	379	63	52	53	49	15	656	69.1%
Pteromalidae	1	6	3	18	29	118	49	224	23.6%
Braconidae	1	1	-	-	3	20	13	38	4.0%
Platygastridae	-	-	-	-	1	4	-	5	0.5%
Eulophidae	-	-	-	-	2	-	1	3	0.3%
Ichneumonidae (others)	-	-	1	-	-	1	1	3	0.3%
Cynipidae	-	-	-	-	-	1	-	1	0.1%
Not identified	-	5	-	-	3	9	2	19	2.0%
Total	47	391	67	70	91	202	81	951	100.0%

Table 4. Adults parasitoids obtained from brassica pod midge sampled with soils in winter wheat fields that followed oilseed rape in 2012 and 2013.

		Assesse (2012)	Buzet (2012)	Ermeton (2013)
Cocoons harvested		1260	3030	101
Rearing success		455 (36%)	255 (8.4%)	61 (60.4%)
Unparasitized (*Dasineura brassicae*)		429	103	64
Parasitized		26 (5.1%)	152 (59.7%)	3 (4.7%)
Platygastridae	*Platygaster subuliformis*	6 (7.7%)	2 (1.3%)	0
Ceraphronidae	*Aphanogmus abdominalis*	9 (34.6%)	23 (15.1%)	1 (33.3%)
	Ceraphron serraticornis	0	124 (81.6%)	0
	Ceraphron insularis	1 (3.8%)	0	0
	Ceraphron bispinosus	7 (26.9%)	3 (2.0%)	1 (33.3%)
	Total	17 (65.4%)	150 (98.7%)	2 (66.7%)
Eulophidae	Not identified	3 (3.8%)	0 (0.0%)	1 (33.3%)

Discussion

The first results of the research recently initiated in winter oilseed rape in Belgium have highlighted the presence and the diversity of parasitoid hymenoptera. In some cases, high parasitism rates were obtained (e.g. pollen beetle larvae > 50% in several fields at several occasion, brassica pod midges in one field, etc.) and new species were found, indicating that this subject has been poorly studied. Parasitoid hymenoptera could be potentially a possibility for IPM in oilseed rape. There was however a great variability between fields and in most of the case, the parasitism levels were low.

A first rough analysis of two parameters that are known to have an impact on pollen beetle parasitism, the landscape diversity and the tillage systems, did not put in evidence differences between objects, but the analysis was only performed on a limited set of data. This study will be continued and further data will be obtained the next years.

The highest larval pollen beetle parasitism level was observed in a field that did not received insecticides in spring (Freyr) and the lowest parasitism rates were in fields that received one or two insecticides (Ernage, Temploux). Even if the data provided by the farmers have not been entirely compiled and analyzed, it seemed that the insecticides regimes (products, timing of the application) could be the most or one of the most parameter interfering with the parasitoid hymenoptera, by limiting their activity.

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Pyrethroid resistance of oilseed rape pest insects in Germany

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Abstract: Pyrethroid resistance of pollen beetle (*Meligethes aeneus*) has been monitored in Germany since 2005 using biotests (Adult-Vial-Test). Since then resistance has spread all over Germany and its intensity has increased. Besides pollen beetles, other pest insects such as weevils and flea beetles were also analysed for pyrethroid resistance. In the German region with the longest tradition and intensity of oilseed rape production, pyrethroid resistance of *Psylliodes chrysocephala* and *Ceutorhynchus obstrictus* with resistance factors of up to 81 and 140 was detected in 2008 and 2010, respectively. This sensitivity change in the biotest was in line with control problems in the field.

Key words: Pyrethroid resistance, oilseed rape, pollen beetle (*Meligethes aeneus*), cabbage stem flea beetle, (*Psylliodes chrysocephala*), cabbage stem weevil (*Ceutorhynchus obstrictus*)

Acknowledgement: Part of the work was funded by the BLE for the Federal Ministry of Food, Agriculture and Consumer Protection and by the UFOP.

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Effect of two different insecticides on the reproduction of pollen beetles in field tests

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Abstract: One of the most important pests of oilseed rape is the pollen beetle (*Meligethes aeneus* F.). Beetles emerging from overwintering sites in spring immigrate to oilseed rape crops and feed on the buds to get access to pollen, which results in bud abortion and high yield loss.

In the past 30 years control of pollen beetle in Germany was mainly based on the application of synthetic pyrethroids. The extensive and indiscriminate use of this insecticide class resulted in a high selection pressure on the beetles, ensuing in the formation of resistance, which has spread over many European countries. Replacement of pyrethroids by insecticides with other mode of action is limited as only few alternative active substances are available.

One of these alternative substances is the neonicotinoid Biscaya (active ingredient thiacloprid). To test the effect of Biscaya and of the pyrethroid Karate Zeon (active ingredient lambda-cyhalothrin) on the reproduction of pollen beetles, a field trial was carried out near Braunschweig. The field trial was divided into control plots without insecticide application, and plots sprayed on different dates with Biscaya or Karate Zeon. Shortly before and after application, the number of beetles was counted in the different plots. Additionally the number of eggs per bud and the number of larvae dropping to the ground for pupation was recorded. Furthermore samples of adult pollen beetles were analyzed for resistance to pyrethroids and sensitivity to thiacloprid by using the Adult-Vial-Test.

Key words: Pollen beetle, *Meligethes aeneus*, insecticide, efficacy

Acknowledgement: Part of the work was funded by the UFOP.

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A study under semi-field conditions on the efficacy of insecticides against *Meligethes aeneus* F. – Methodical approach and analysis

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Abstract: Oilseed rape (*Brassica napus* L.) is one of the most important oilseed crops in the world. One of the major pests is the pollen beetle (*Meligethes aeneus* F.) (Nitidulidae). Under certain conditions the beetle can cause yield losses of up to 100%. One contributing factor is the decreased sensitivity to pyrethroid insecticides which has been reported from several European countries. There are two methods for insecticide susceptibility tests: insecticides can be tested in field trials or in the laboratory using the ‘glass vial testing method’. The presented approach is a semi-field method coming with precise statements of the reactions of the beetles to insecticides under realistic field conditions. The method is implemented to study the efficacy of insecticides with different mode of actions. Pollen beetle populations were collected from untreated fields in Saxony-Anhalt, Germany. Six insecticides with different mode of actions were sprayed in the field. Treated plants were cut and brought into the greenhouse on nine sampling occasions. Each plant was placed into a perforated plastic bag with 10 beetles. The vitality of the beetles was observed after two and five days following inoculation. To describe the efficacy of insecticides the beetles were divided into three categories (alive, damaged and dead). These observations were interpreted as a realization of classified ordered categorical random variables. For the analysis we used a threshold model (generalized linear model). The dependence of the observations due to repeated observations on the same plant was accounted for by a random plant effect. The comparison of the different insecticides and their significance test was made by using the marginal expectation values. For the computational implementation, we used the procedure NL MIXED (SAS 9.3).

Key words: oilseed rape, pollen beetle, semi-field experiment, susceptibility test

Introduction

Oilseed rape (*Brassica napus* L.) is one of the important oil crops in the world. During its cultivation, a suite of several insect pests may attack the plants. One of the most important is the pollen beetle (*Meligethes aeneus* F.) (Nitidulidae). Under certain conditions the beetles can cause yield losses of up to 100% (Slater *et al.*, 2011). One contributing factor to such devastating loss is a decreased sensitivity to the pyrethroid insecticides used for control which has been reported from several European countries (Zimmer & Nauen, 2011).

There are two established methods to test insecticide efficacy a) pyrethroid monitoring in the laboratory using insecticide-coated glass vials and b) in field trial studies. The approach we present here is a method carried out in semi-field conditions with precise statements of the reactions of the beetles to insecticide under realistic field conditions. The method is implemented to study the efficacy of insecticides with different mode of actions. Our study took place in 2010-2013. The first year in 2010 was used to test the experimental design. In

2011, the experimental settings were extended. The results from these two years are explained in two master theses (Bormann, 2011; Kaiser, 2013). The third year was carried out by new bachelor students with some modifications. Here we present results from 2011.

Material and methods

The experiment took place during the pollen beetle damage-susceptible bud stage before flowering. Pollen beetles were collected from untreated oilseed rape fields in Saxony-Anhalt, Germany. They were stored during the experiment in a climatic chamber at 4 °C, within perforated plastic bags with some oilseed rape inflorescences for food and a small filter paper to collect any condensation.

The different insecticides were sprayed on a site at Spickendorf near Halle (Saale), Germany. Each insecticide was sprayed once on a single plot (4 x 20 m) in an oilseed rape field at the bud stage. In 2011, the following insecticides with different mode of actions were used: pyrethroids (Karate and Trebon), neonicotinoids (Biscaya and Mospilan), oxadiazine (Avaunt), and pymetrozine (Plenum). Ten treated plants were randomly selected and cut at nine dates and brought into the greenhouse. The first plants were first collected 2 h after spraying and then on eight consecutive occasions. The plants were placed immediately in a measuring cup with 200 ml water to keep them fresh. Each plant was placed in a perforated plastic bag along with 10 pollen beetles. The beetles were kept for five days in this simulated habitat. After two and five days the pollen beetles were observed and each was assigned to one of three categories (alive, damaged or dead). Pollen beetles were classified as damaged when they showed uncoordinated movements, if they were not able to spread their wings properly and/or were unable to walk straight. These observations were interpreted as a realization of classified ordered categorical random variables. For the analysis we used a threshold model (generalized linear model). The dependence of the observations due to repeated observations on the same plant was accounted for using a random plant effect. The comparison of the different insecticides and their significance test were made of the marginal expectation values. For the computational implementation, we used the procedure NL MIXED (SAS 9.3).

Results and discussion

As an example of the development of the novel susceptibility testing method, the results with Biscaya (active compound Thiacloprid) are presented here from 2011; experimental conditions were good, as in the control treatment 92-100% of the beetles were alive and undamaged throughout this experiment (Figure 1).

In 2011, two days after application, the Biscaya treatment showed only 28% undamaged beetles and 72% beetles were characterized as damaged or dead (Figure 2). The maximum effect was reached on the third day after application when about 80% of the beetles were damaged or dead in the first observation. The maximum of damaged and dead beetles with 87% was reached six days after application in the second observation.

During the entire bud stage the efficiency of the insecticides could be observed with this methodical approach. This experiment simulates the continious immigation of pollen beetle to the fields because the beetles had only contact to the insecticides from that moment when they were placed on the plant. Immigation into the oilseed rape crop happens over a prolongued period during the entire preflowering period of the crop and is dependant on weather conditions. This experimental setup provides results at the interface between research and practice. The insecticide application in field and the time graduation of the plant sample shows the real reduction of the efficacy. The reduction is caused by the growth of the plants and the weather conditions.

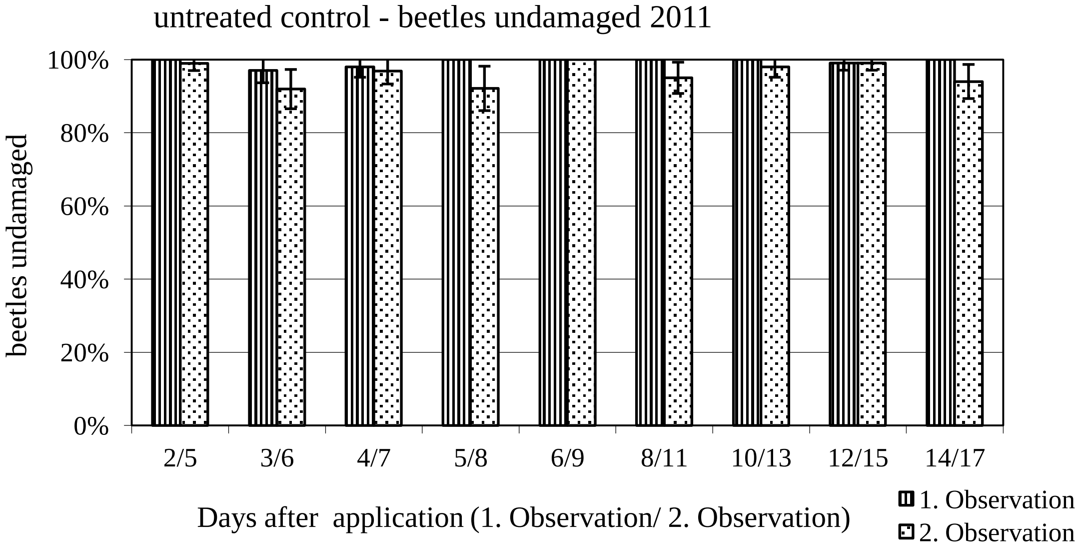

Figure 1. Undamaged beetles in the untreated control in 2011. Plants were collected on 9 occasions following application of the treatment. From each sample, beetles were observed twice, 2 and 5 days after inoculation (Observation 1 and Observation 2, respectively).

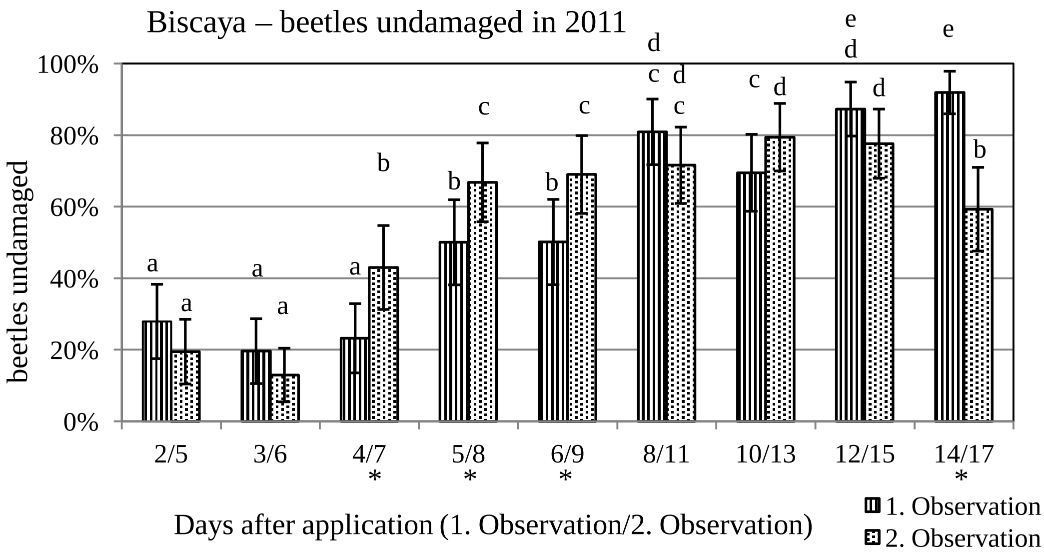

Figure 2. Undamaged beetles in the Biscaya treatment in 2011. Plants were collected on 9 occasions following application of the treatment. From each sample, beetles were observed twice, 2 and 5 days after inoculation (1. Observation and 2. Observation, respectively).
* = significant differences between the 1. Observation 1 and 2
Treatments with different letters show significant differences

Acknowledgements

We thank the colleagues from Bayer CropScience for financial support. We gratefully acknowledge Prof. Dr. Volkmar for her great support during the entire study. We also thank Prof. Dr. Spilke for analytical support.

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A large field trial to assess the short-term and long-term effects of 4 insecticides used to control the pollen beetle on parasitic hymenoptera in oilseed rape

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Abstract: A large-scale field trial was performed in spring 2013 to assess the effects of Plenum (pymetrozine), Mavrik 2F (tau-fluvalinate), Biscaya (thiacloprid) and Pyrinex (Chlorpyriphos-ethyl) used to control the pollen beetle *Meligethes aeneus* (F.) (Col.; Nitidulidae) on the populations of pests and beneficial arthropods in winter oilseed rape. The insecticides were applied at their commercial rate just before flowering on large strips of oilseed rape (30 m x 200 m), divided into four plots of 50 m x 30 m. A strip was left untreated as control. Insects were sampled by plant beating methods and sweep netting the day after treatment and thereafter weekly up to 50 days after product application. The direct effects of the products were assessed on adult pollen beetle (target pest), adult cabbage seed weevil *Ceutorrhynchus obstrictus* (Marsham) (Col.; Curculionidae) (secondary pest) and adult parasitic hymenoptera associated with these insects (Tersilochinae and Pteromalidae). In the context of IPM, long term effects were assessed on pollen beetle larvae to determine their number, the parasitism rate and to estimate the balance of parasitic hymenoptera/pollen beetle that could be produced by the field for the next season.

Biscaya, Pyrinex and Mavrik 2F were effective in controlling the adult pollen beetle population and also had activity on cabbage seed weevil, despite the fact that this pest only occurred 2 or 3 weeks after the application of product. Plenum was only effective in controlling the pollen beetle population 1 day after treatment and had no significant impact on cabbage seed weevil.

All the insecticides tested had a significant impact on the population of adult parasitic hymenoptera compared to the control. Plenum had limited effects while the other insecticides reduced by 59-72% the numbers captured in the sweep net samples. The main effects were observed on adults of the Tersilochinae family that are mainly specialised in the parasitism of pollen beetle larvae. Biscaya also had a significant impact on numbers of Pteromalidae caught, a family containing species specialised in the parasitism of weevils, despite the 4-week delay between the day of the treatments and the first arrival of these hymenoptera in the crop.

Biscaya significantly reduced the parasitism rate of the pollen beetle larvae, which was reduced to less than 15% compared to 43.2% in the control. Pyrinex also decreased the parasitism rate with only 23% larval parasitism, but the difference was not significant. Biscaya and, to a lesser extend Pyrinex, reduced the balance of parasitic hymenoptera: pollen beetle, while Plenum and Mavrik 2F led to the same ratio as the untreated control. These results suggest that the regular use of Biscaya and/or Pyrinex on a large scale before flowering is favourable to the long term development of pollen beetle populations by negatively impacting the populations of their parasitoids, despite their good short-term efficacy to control this pest.

Key words: insecticide, side effects, pollen beetle, parasitic hymenoptera, parasitism

Introduction

The pollen beetle has become one of the most important pest problems in oilseed rape in Western Europe during these last years, mainly by the apparition of population resistant to insecticides (Thieme *et al.*, 2010). Now, in the context of the development of IPM, a specific attention is given to the possible natural control of this pest by its natural enemies. Specific hymenoptera belonging to the Tersilochinae subfamily (Hym.; Ichneumonidae) appeared to be as the key pollen beetle natural enemy (Ulber *et al.*, 2010a), with larval parasitism rates across Europe in the range of 25-50% in unsprayed crops, or even higher in some cases. However, these beneficial insects are exposed to several insecticides applied in spring in oilseed rape, when they are foraging for host location or in the crop where the adults emerged after the winter spent in the soil, mainly wheat drilled after the oilseed rape.

Several insecticides still in use in oilseed rape have already been assessed on pollen beetle parasites. Most of the products still in use were highly toxic for the adults, with reduction of the adult populations regularly higher than 50% compare to the untreated control. These products had also a negative impact on pollen beetle parasitism (Halden, 2004, Ulber *et al.*, 2010b). Compared to other pyrethroids insecticides, tau-fluvalinate (Mavrik 2F) appeared as the less toxic product, but the effects cannot be considered as negligible.

Due to resistance problems, several other insecticides were recently registered in oilseed rape, as chlorpyriphos-ethyl and thiacloprid. These product have not yet been assessed on Tersilochinae species, but at each time they were tested on a parasitic hymenoptera species, they were highly toxic (Van de Veire & Tirry, 2003; Medina *et al*., 2008; Jansen, 2010). Another insecticide with a totally different mode of action, pymetrozine, known to be effective mainly on aphids, has also been recently authorized in oilseed rape. This product was considered as selective for a large set of beneficial arthropods (Jansen *et al.*, 2011), including the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) (Hym.; Aphidiidae), often used as an indicative species for the parasitic hymenoptera group. The aim of this work was to assess in the field the effects of these new products and of Mavrik 2F on parasitic hymenoptera, with specific assessments on pollen beetle parasitism.

Material and methods

Test products

The test was implemented in a commercial oilseed rape field cropped under the current agricultural practices for the area. The field was located at Onhaye (Ferme de Lenne, 50°13'24.82''N, 4°52'36.13''E) and 3.00 ha of the total field site (17.0 ha) was dedicated to the trial. No insecticides were applied before and during the tests, except the test products, while the rest of the field was treated on the 24th April with Mavrik 2F. The entire field was treated with a fungicide during flowering (Cantus 0.5 kg/ha, Boscalid 50% WG). The field site was divided into 5 strips of 30 m x 200 m that received one of the 4 insecticide treatment or was left untreated (control). Each strip was divided into four plots of 30 m x 50 m, each plot being considered as a replicate. All samples and visual counts were done in the 20 m x 40 m central part of each plot. The field was nearly completely surrounded by woods.

The test insecticides were: Plenum (Pymetrozine, 500g/l, WG), applied at 150 g/ha, Biscaya (Thiacloprid, 240 g/l, OD) applied at 300 ml/ha, Mavrik 2F (tau-fluvalinate, 240 g/l, EW) applied at 200 ml/ha, and Pyrinex (Chlorpyriphos-ethyl, 240 g/l CS) applied at 750 ml/ha. The doses correspond to the commercial recommended rates. The insecticides were applied on the 26th of April at the end of the bud stage (GS 3.6-3.7), on basis of a volume of 200 l spray mixture/ha.

Sampling techniques and organization

Different sampling techniques were used during the experiments, according to the main target insects and the climatic conditions: plant beatings (into plastic trays and funnels) and sweep netting methods. The details of the sampling methods used on each assessment dates are given in Table 1.

Table 1. Schedule of the sampling methods used. DAT= days after treatment, DBT= days before treatment.

Sampling method	DBT5	DAT1	DAT8	DAT13	DAT22	DAT29	DAT36	DAT43	DAT50
Tray beating	X	X	X	X					
Funnel beating					X	X			
Sweep net		(X)[1]	X	X			X	X	X

[1] only in the control to detect first adults of parasitic hymenoptera. Not found at this date and not performed in the other plots.

Before the first occurrence of pollen beetle larvae, plant beating was used to assess the adult pollen beetle population. The terminal part (stem and flower buds) of 20 plants randomly selected in each plot were shaken just above a plastic tray (40 x 30 x 8 cm). The insects that fell onto the trays (mainly pollen beetle) were immediately identified, counted then released. Several weevils were also counted and recorded.

When the first larvae of pollen beetle were detected, the “funnel beatings” method was used for sampling, to allow the collection of pollen beetle larvae. This method was also used to assess populations of parasitic hymenoptera when sweep net sampling was not possible, due to wet weather conditions. During ‘funnel beating’, 20 terminal racemes were randomly selected from each plot and shaken just above a plastic funnel (Ø 30 cm) placed into a plastic bottle (250 ml). The insects that fell on the funnel were rinsed with water (+ commercial soap) to collect them into the plastic bottle and they were returned to the laboratory for counting, identification and determination of the parasitism rates for the pollen beetle larvae.

For sampling of parasitic hymenoptera, reinforced nets (Ø 35 cm) were used, with 10 side-to-side sweeping moves (sampling of around 3-4 m^2). The sweep nets were emptied over a funnel, identical to those used for the funnel samplings. Adults and larvae of pollen beetle and adult weevils were also collected with this method, with results comparable to the funnel samplings (based on a comparison made in the control).

Sampling started 5 days before the application of products and were performed 1 day after the product applications (DAT1) and then at 7 (± 1 day) intervals until 50 DAT, corresponding to the end of flowering.

Analysis

The short term effects, that concerned the field where the products were applied and the longer-term effects that concern the pest: beneficial ratio for the following years were both considered.

The short-term effects were assessed on adults of pollen beetle (target pest), adults of seed weevils (secondary pest) and adults of the parasitic hymenoptera, identified at the family level for Tersilochinae (mainly pollen beetle larval parasitoids) and Pteromalidae (mainly seed weevil larval parasitoids).

The long-term effects were assessed on pollen beetle larval populations and pollen beetle larval parasitism. These two results were used to estimate the balance between the numbers of adult parasitoids and adult pollen beetles produced by the treated area for the next growing season. These were calculated as follows for each plot:

$$R = (Lp/Lnp) * 100$$

R = balance; Lp = pollen beetle larvae parasitized sampled in the corresponding plot; Lnp = pollen beetle larvae unparasitized sampled in the corresponding plot

An R balance value of 100 indicates that the plot will produce the next year the same number of adult parasitoids as adult pollen beetles, taking as working hypothesis that all larvae are viable, and would produce an adult for the next season. This hypothesis is probably false as several factors can affect the success of the larval development, especially for the parasitoids which overwinter in the soil and are susceptible to tillage effects, but this method is simpler than the direct assessment of the emergence of adult beetles and parasitoids (Nilsson, 2010) However, as the factors affecting the larval development of both pollen beetle and parasitoid are not dependent on the experimental treatments, this method provides a useful rough assessment to compare the effects of different insecticides regimes.

Statistical analysis

The data were analysed for differences using one-way Anova tests (R software). Pair-wise comparisons were performed using Tukey (multiple comparisons) and Dunet-test (comparison to the control for efficacy results) at the 0.05 level. Data were log transformed if necessary to normalise the distribution before analysis.

Results

Adults of pollen beetle

The number of adult pollen beetles sampled at the different dates is given in Table 2. All treatments were effective one day after product application, with a reduction of pollen beetle population ranging from 82-100%. Eight days after treatments, Mavrik 2F, Biscaya and Pyrinex reduced significantly the populations, with a range of 76-100% efficacy. Plenum was no longer effective with only 20% reduction. At DAT13, only Pyrinex reduced significantly the pollen beetle populations compared to the control. It must be noted that the populations were reduced in the control on this date and that the populations were low in all treatments with proportionally higher variability. Some adult pollen beetles were also sampled from DAT22 to DAT50 but these results were not taken into consideration as numbers were very low (mean of 1.1 pollen beetle/assessment in the control) and the crop was no longer sensitive to pollen beetle, due to having progressed well into the flowering growth stages (during which time the crop is no longer susceptible to pollen beetle damage).

When the assessments carried out at the different dates were pooled together, the results indicated that all the treatments significantly reduced pollen beetle population when directly compared to the control, but Plenum was less effective than the 3 other insecticides.

Cabbage seed weevil

The cabbage seed weevil was not the target of the trial for the selection of products and the timing of the application, but the populations sampled at the same time as the pollen beetle were high and allowed us to assess the impact of the insecticides on this species. The results are listed in Table 3. The data have been pooled in two groups to facilitate statistical analysis: the early populations (DAT1-13), from bud stage to the beginning of flowering with a first population peak at DAT13 (9.0 weevils in the control) and the later population, when the plants were flowering (DAT22-50), with a peak at DAT36 (16.25 weevils in the control). The results showed that all products except Plenum had an impact on the cabbage weevil population; even if the effects were lower than for the Pollen beetle.

Table 2. Mean number of adult pollen beetles collected ± sd at different dates after treatments (DAT) and efficacy compared to control.

	DAT1	DAT8	DAT13	Total
Control	16.25 ± 4.2 a	11.25 ± 4.6 a	6.50 ± 2.1 ab	34.00 ± 2.2 a
Plenum (pymetrozine)	1.50 ± 1.9 bc	9.00 ± 3.9 ab	9.00 ± 6.7 a	19.50 ± 12.1 ab
Mavrik 2F (tau-fluvalinate	1.00 ± 0.8 bc	1.75 ± 2.1 cd	3.00 ± 1.8 ab	5.75 ± 3.3 cd
Biscaya (thiacloprid)	1.25 ± 0.5 bc	2.00 ± 1.4 cd	3.25 ± 1.9 ab	6.50 ± 2.6 c
Pyrinex (chlorpyriphos-ethyl)	0.50 ± 1.0 bc	0.50 ± 1.0 cd	2.00 ± 1.2 bc	3.00 ± 1.6 cd
Control	*- a*	*- a*	*- a*	*- a*
Plenum	*91% b*	*20%a*	*-38% a*	*43% b*
Mavrik 2F	*94% b*	*84% b*	*54% a*	*83% b*
Biscaya	*92% b*	*82% b*	*50% a*	*81% b*
Pyrinex	*96% b*	*96% b*	*69% b*	*91% b*

GLM, One-way Anova (p = 0.05 level) followed by pair-wise Tukey test for population (results followed by the same letter are not statistically different) and Dunet test for efficacy (a = not different to control, b = different to control).

Table 3. Mean number of adult seed weevils collected ± sd at different dates after treatments (DAT) and efficacy compared to the control.

	DAT1-13	DAT22-50	Total
Control	13.00 ± 0.8 a	29.00 ± 13.3 a	42.00 ± 13.5 a
Plenum (pymetrozine)	9.25 ± 2.6 b	22.50 ± 9.4 a	31.75 ± 9.7 ab
Mavrik 2F (tau-fluvalinate	3.50 ± 0.6 bcd	17.00 ± 13.4 bc	20.5 ± 13.6 bc
Biscaya (thiacloprid)	3.25 ± 1.7 cd	8.00 ± 5.2 c	11.25 ± 4.0 c
Pyrinex (chlorpyriphos-ethyl)	7.25 ± 2.1 bc	11.75 ± 4.4 bc	19.0 ± 6.1 c
Control	*- a*	*- a*	*- a*
Plenum	*29% a*	*22% a*	*24% a*
Mavrik 2F	*73% b*	*41% b*	*51% b*
Biscaya	*73% b*	*75% b*	*72% b*
Pyrinex	*55% b*	*44% b*	*59% b*

GLM, One-way Anova (p = 0.05 level) followed by pair-wise Tukey test for population (results followed by the same letter are not statistically different) and Dunet test for efficacy (a = not different to control, b= different to control).

Parasitic hymenoptera

The numbers of adult parasitoids sampled during the experiments are listed in Table 4. Results of the different assessments have been pooled for statistical analysis. The two main families of hymenoptera found were Tersilochinae (mainly endoparasitoids of pollen beetle larvae) and Pteromalidae (mainly exoparasitoids of seed weevil larvae). The Tersilochinae were mostly sampled between DAT1 and 13, with more than 60% of the adults sampled at this last date in the control. The Pteromalidae were collected later with a first record at DAT29. These dates were linked to the presence of the hosts (pollen beetle larvae and seed weevil larvae). The identification of these two families to the species level is planned. A few adults of the Braconidae *Diospilus capito* (0 to 0.5/sample in total) were also identified from the samples. As this species is also associated with pollen beetle larvae, these records were added to the results of the Tersilochinae. A few adult parasites of other families, probably parasitoids of brassica pod midge, were also identified but not analysed separately, as the populations were very low (0.0 to 0.5 specimens by object in total).

Table 4. Mean number of adult parasitic wasps collected ± sd between DAT1 and DAT50 and reduction of the populations compared to the control. Tersilochinae (pollen beetle parasitoids), Pteromalidae (seed weevil parasitoid) and total, including the other families.

	Tersilochinae	Pteromalidae	Total
Control	36.00 ± 9.8 a	7.75 ± 2.2 a	43.75 ± 10.6 a
Plenum (pymetrozine)	24.25 ± 6.2 b	6.00 ± 3.2 a	30.75 ± 5.6 b
Mavrik 2F (tau-fluvalinate	10.50 ± 6.6 c	5.25 ± 2.4 a	16.25 ± 6.8 c
Biscaya (thiacloprid)	10.00 ± 7.0 c	3.25 ± 2.1 b	13.50 ± 8.7 c
Pyrinex (chlorpyriphos-ethyl)	6.50 ± 7.0 c	5.25 ± 1.5 a	12.25 ± 8.5 c
control	*- a*	*- a*	*- a*
Plenum	*32.6% b*	*23% a*	*30% b*
Mavrik 2F	*70.8% b*	*32% a*	*63% b*
Biscaya	*72.2% b*	*58% b*	*69% b*
Pyrinex	*81.9% b*	*32% a*	*72% b*

GLM, One-way Anova (p = 0.05 level) followed by pair-wise Tukey test for population (results followed by the same letter are not statistically different) and Dunet test for efficacy (a = not different to control, b = different to control).

The adults of Tersilochinae were affected by all the treatments. Plenum was the least toxic product for this family, with a reduction of 32.6% compared with the control. The other products have a similar pattern with a reduction of adult populations of 70-80% compared to the control.

Only one insecticide, Biscaya, significantly affected the Pteromalidae adult population when directly compared to the control.

The total adult parasitic hymenoptera population reductions followed the same trends as the Tersilochinae results, with a limited effect of Plenum and a more important effect of all other treatments. This could be explained by the Tersilochinae records, which were the most abundant group in the samples.

Long term effects – Pollen beetle larvae and parasitic hymenoptera larvae
The number of pollen beetle larvae and their parasitism rates are listed in Table 5. The long term effects of the product, assessed by estimating the number of parasitoids produced for 100 pollen beetles is also included in this table.

Table 5. Mean number of pollen beetle larvae collected ± sd between DAT1 and DAT50, parasitism rate and balance adult parasite/pollen beetle.

	Total larvae	% parasitism	Adult parasitoid/100 pollen beetle
Control	122.3 ± 60.8 a	43.2% ± 8.2% a	79.1 ± 27.9 a
Plenum (pymetrozine)	92.5 ± 50.5 a	34.5% ± 5.2% a	53.4 ± 11.4 a
Mavrik 2F (tau-fluvalinate	30.3 ± 14.2 b	32.3% ± 6.6% ab	48.7 ± 15.0 ab
Biscaya (thiacloprid)	19.8 ± 18.1 bc	14.5% ± 4.4% b	17.2 ± 6.1 c
Pyrinex (chlorpyriphos-ethyl)	8.5 ± 1.30 c	23.3% ± 8.0% ab	31.4 ± 13.0 bc

GLM, One-way Anova (p = 0.05 level) followed by pair-wise Tukey test (results followed by the same letter are not statistically different).

All products except Plenum significantly reduced the number of pollen beetle larvae found in the treated plots. Pyrinex was the most toxic product. The analysis of the parasitism rate of these larvae indicated that all products except Biscaya had no significant impact on the parasitism rate of the pollen beetle larvae compared with control plots. Pyrinex also had a large effect on the parasitism rates but the differences to the control were only marginally significant ($0.05 < p < 0.10$). The ratios of adult parasitoids that will be produced by the treated plots per 100 pollen beetles confirm these observations, with a significant negative impact observed for Biscaya and Pyrinex, compared to the control and no differences between the control, Mavrik 2F and Plenum.

Discussion

The products assessed during this study have shown different effects on pest and beneficial insect populations. In terms of adult pollen beetle control, all treatments have good efficacy directly after application. Plenum had however limited effect one week after application while the other products were still effective. In the test conditions, with the treatment applied just before flowering, all products, except perhaps Plenum, can be used to control adult pollen beetle.

Most of the insecticides applied to control pollen beetle before flowering had also an impact on adults of the cabbage seed weevil, even if the first significant population of this insect was detected until DAT13 in the control. Plenum was ineffective against the seed weevil while Biscaya, Mavrik 2F and Pyrinex reduced the population in the range of 49-70% compared to the control. These values may not perhaps be sufficient for effective chemical control of this pest, but timing of treatment was not ideal. Our data indicate the residual activity of treatments made 2 weeks before arrival of the pest are not negligible and perhaps sufficient for control in crops with a low seed weevil pressure.

The effects of the products on adult parasitic wasps were high for most of the products, with a reduction of 60-70% of the populations. Plenum was the only product with limited effects (30%) when compared to the control. When the hymenoptera families were identified and related to their expected hosts, the main effects were observed on adults of Tersilochinae, mostly associated to pollen beetle. The effects on Pteromalidae, which are mainly associated to the cabbage seed weevil, were limited and non-significant for all of the products, except Biscaya. As these hymenoptera arrived later in the season (first observation 29 days after the application of the insecticides), these results indicated that Plenum, Mavrik 2F and Pyrinex, applied soon before flowering to control pollen beetle, had no significant impact on the main cabbage seed weevil parasitoid family. The only exception was Biscaya, with a significant impact on these insects despite the 4-week delay between the application and the colonization of the crop by these beneficials.

The effects of the insecticides on pollen beetle larvae indicated that most of the products reduced the total number of pollen beetle larvae found on the plants, and the parasitism rates by Tersilochinae were not affected compared to the control with Mavrik 2F and Plenum. However, Biscaya and, to a lesser extent, Pyrinex, had a different effect profile and reduced the parasitism rate of the pollen beetle larvae and the ratio of parasitoids: pest, with significant differences compared to the control. This could indicate that the repeated use of these insecticides could affect the population of beneficial parasitic hymenoptera and should not be recommended in the context of IPM.

These conclusions were, however, only based on one year's trial data and need to be carefully replicated in future experiments.

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Parasitism of pollen beetle, *Meligethes aeneus* F., in different regions of Northern Germany

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Abstract: Hymenopterous parasitoids have the natural potential to regulate populations of pollen beetle (*Meligethes aeneus* F.). The univoltine larval endoparasitoids *Tersilochus heterocerus* and *Phradis spp.* (Hym.; Ichneumonidae) have been reported as key natural enemies of pollen beetle in oilseed rape fields of various European countries. Previous studies have shown that the level of larval parasitism of pollen beetle varies widely between different locations and years. To obtain more information on possible factors causing spatial and temporal variation between the levels of parasitism, crops of winter oilseed rape were selected in four different regions along a transect from west to east of Northern Germany (districts of Diepholz, Uelzen, Flaeming, Oder-Spree), with 3-6 crops in each region. Among other factors, these regions differed with regard to mean annual temperature, mean precipitation and the proportion of arable land grown with oilseed rape.

In the years 2011 and 2012, larval parasitism of pollen beetle was determined in samples collected from plots without insecticide application (1000 m²) within commercial crops of oilseed rape. First and second instar larvae of pollen beetle were collected during full flowering (BBCH 65-69) from a total of 16 and 17 crops, respectively, by beating the main inflorescenses into a tray. Pollen beetle larvae were dissected in the laboratory under a binocular microscope to detect the level of parasitism. Parasitism by *T. heterocerus* and *Phradis* spp. was recorded by assessing dark pigmented eggs and neonate larvae, respectively, separately.

The results showed substantial variation between the levels of larval parasitism of pollen beetle in crops of oilseed rape at closer scales (within regions; distance 0.5-45 km) and between the two years. Total parasitism ranged between 4-25% in 2011 and 1.8-26% in 2012. At larger scales (distance 115-375 km), in 2011 the mean level of parasitism differed between all four regions while in 2012 mean parasitism was on a similar level in the two eastern and the two western regions, respectively. Regarding the relative abundance of parasitoid species, the proportion of *T. heterocerus* and *Phradis* spp. on the total parasitism differed between the four regions but also within the regions between years. Except of the Diepholz region, *T. obscurator* was the predominating parasitoid species in all regions and years. Generally, the data have not provided any indications that larval parasitism of pollen beetle is influenced by regional longitude or spatial scale (distance). Future data analysis will focus on field specific parameters like field size, insecticide application and percentage of oilseed rape grown in the area surrounding the studied fields. Furthermore, data of a third year (2013) will support the upcoming analysis.

Key words: winter oilseed rape, *Meligethes aeneus*, larval endoparasitoids, *Tersilochus heterocerus*, *Phradis* spp., spatial effects

Pollen beetles
(*Meligethes aeneus*)

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IOBC-WPRS Bulletin Vol. 104, 2014
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Olfactometer screening of repellent essential oils against the pollen beetle (*Meligethes* spp.)

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Abstract: Essential oils can have an impact on pollen beetle (*Meligethes spp*.) host plant location behaviour. Lavender oil (*Lavendula angustifolia*) showed the highest repellency value in a previous laboratory study that compared five different essential oils (Mauchline *et al*., 2005). However, lavender oil is one of the most expensive essential oils – a fact that could seriously hamper on-farm implementation of this strategy. To find a cheaper essential oil with comparable efficacy to lavender oil, we compared the essential oils of *Mentha arvensis, Eucalyptus globulus, Melaleuca alternifolia, Citrus sinensis, Citrus paradisi, Citrus limon, Juniperus mexicana, Abies sibirica, Illicium verum, Gaultheria procumbens, Cymbopogon flexuosus, Syzygium aromaticum,* and *Litsea cubeba* using a Y-tube-olfactometer. Essential oils were diluted 1:10 in acetone and 40 µl was applied to a 3.1 cm-diameter filter paper. Filter papers were placed in the odour containers of the olfactometer together with a flower cluster of spring oilseed rape with 5 open flowers and 10-15 buds. The control treatment involved filter papers treated only with acetone. Hungry pollen beetles were released individually into the olfactometer. The beetles' choices were recorded. Flowers and essential oils were changed between replicates. Six replicates with six beetles each were conducted. Highest repellency values were obtained for *Mentha arvensis, Cymbopogon flexuosus,* and *Litsea cubeba.*

Key words: *Meligethes aeneus, Meligethes viridescens*, organic agriculture, essential oil, lavender, cornmint, lemongrass, repellent

Introduction

Organic agricultural methods to control pollen beetle (*Meligethes* spp.) are limited. Although effective insecticides are available for organic producers (e.g. Spinosad), their use is often restricted by guidelines of producers associations. In Swiss organic production (Bio Suisse), as well as in Swiss IPM-production (IP-SUISSE) the use of insecticides in oilseed rape is not permitted. Therefore, alternative non-insecticidal methods to control pollen beetles are needed. Experiments in the UK showed that essential oils can have an impact on pollen beetle (*Meligethes aeneus*) host plant location behaviour (Mauchline *et al*., 2005; Mauchline *et al.*, 2008; Mauchline *et al.*, 2013). In a laboratory study that compared five different essential oils, lavender oil (*Lavendula angustifolia*) showed the highest repellency value (Mauchline *et al*., 2005). However, lavender oil is relatively expensive, and cost is an impediment for farms to adopt it as a repellent. We compared 15 different essential oils using a Y-tube-olfactometer to see if any of lower cost would have similar efficacy.

Material and methods

Pollen beetles
The pollen beetles used in all experiments were collected in an untreated winter oilseed rape field in north-western Switzerland. Beetles were starved for 40 hours before starting the experiment.

Flowers
Glasshouse-grown spring oilseed rape flowers (cv. Hero) were used as odour source. Flower clusters with 5 open flowers and approximately 10-15 closed buds were cut from plants imediately before starting the experiments and were permanently supplied with water.

Experimental aparatus
The experiments were conducted using a Y-tube-olfactometer (Belz *et al.*, 2013). Odour sources were placed in the odour containers of the olfactometer. Pollen beetles were released individually into the olfactometer using flexible forceps. Observations started as soon as the beetle crossed the 'start' line 1.5 cm from the release point; 10 cm distance to the Y-junction, and stopped as soon as the beetle crossed the 'finish' line (4 cm behind the Y-junction). The choice of the beetle was recorded. The beetle was removed from the olfactometer for determination of species (*M. aeneus, M. viridescens*) and sex. Beetles that failed to cross the finish line within 90s after crossing the first line, were removed from the olfactometer and were not included in the analysis. Each replicate consisted of six responding pollen beetles. Odour sources (= flowers and repellent odours) were replaced after each replicate. Six replicates (= total 36 beetles) were conducted per treatment. The sequence of the tested substances was randomized between each replicate to avoid influence of the time of day. Experiments were conducted between 07:30 and 16:30 h. The experimental room was kept at 22 ± 3 °C, 50 ± 10% relative humidity.

Essential oils
The following essential oils, supplied by qualiessentials GmbH (Germany), were used in the experiments: cornmint oil (*Mentha arvensis*), orange oil sweet (*Citrus sinensis*), wintergreen oil (*Gaultheria procumbens*), lemongrass oil (*Cymbopogon flexuosus*), eucalyptus oil (*Eucalyptus globulus*), fir needle oil (*Abies sibirica*), lemon oil (*Citrus limon*), tea-tree oil (*Melaleuca alternifolia*), clove oil (*Syzygium aromaticum*), star anise oil (*Illicium verrum*), grapefruit oil white (*Citrus paradisi*), Texas cedarwood oil (*Juniperus mexicana*), *Litsea cubeba* oil (*Litsea cubeba*), lavender oil (*Lavendula angustifolia*). In addition to the essential oils, the product Heliosol (Omya Agro, Switzerland) was tested. Heliosol is a pine oil based product used as wettening and sticking agent for plant protection purpose. Essential oils and Heliosol were diluted 1:10 in acetone and 40 µl was applied to a 3.1 cm^2 filter paper (MN713, Macherey-Nagel, Germany). After 30 minutes, when the acetone had evaporated, the filter papers were placed together with a flower cluster in the odour containers of the olfactometer. The control used filter papers treated only with acetone with a flower cluster.

Statistical analysis
Repellency values (RV) were calculated per replicate according to Mauchline *et al*. (2005): [RV = number of beetles on the untreated flower / (number of beetles on the untreated flower + number of beetles on the treated flower)]. In order to test if the essential oils had a significant effect compared to the "untreated" control, a Wilcoxon signed rank test was performed to test whether mean RV was significantly different from 0.5. In order to compare

the efficacy of different essential oils, the RV were [arcsin√(x)] transformed. Normality of data and homogeneity of variance were tested. RV were compared by an ANOVA followed by a Tukey HSD post hoc tests ($\alpha = 0.05$).

Results and discussion

Behaviour of beetles in the olfactometer

When released into the olfactometer, the pollen beetles needed a few seconds to get on their feet and to orient themselves towards the airflow. Once they started to walk, they moved forward until they reached the junction of the two arms. Irritated either by the light, which was placed directly above the junction or by the disturbance in the airflow, most beetles stopped or walked a vertical looping in the junction. After a few seconds, they started walking into one arm of the olfactometer, usually at a rapid pace. On average, it took the beetles 39.8 ± 0.6 s to cover the distance (14 cm) from the ‘start’ to the ‘finish’ line. Beetles rarely moved back into the other olfactometer arm. A total of 772 beetles were released into the olfactometer. Out of these beetles, 232 individuals (= 30%) failed to cross the finish line within 90 s after crossing the first line. They were removed and not included in the analysis. A total of 540 beetles were responsive. Beetles showed best reaction early in the morning. This might be due to the daily activity pattern of pollen beetles: food foraging behaviour is possibly stronger in the morning. Out of the 540 responding beetles, 474 individuals were *Meligethes aeneus*, 66 individuals (= 12.2%) were *Meligethes viridescens*. The sex ratio (M:F) of 0.47 was identical for both species.

Pollen beetle choice in the olfactometer

Ten out of the 15 tested essential oils in a 1:10 dilution significantly repelled the pollen beetles and showed a repellency value (RV) significantely greater than 0.5 (Table 1; Wilcoxon signed rank test, $p < 0.05$). The essential oils from cedarwood, orange, wintergreen, eucalyptus and lemon did not have a significant effect on pollen beetle choice. The RV was not significantly different from 0.5 (Wilcoxon signed rank test, $p > 0.05$). However, all tested essential oils had a mean RV > 0.5. Thus, none of the tested essential oils was attractive for the pollen beetles. Cornmint had the highest RV (1.00): none of the beetles chose the olfactometer arm with cornmint essential oil. Lemongrass and *Litsea* essentail oils, as well as the pinolene based plant protection product Heliosol also showed high repellency values of 0.92. Lavender oil was less repellent with an RV of 0.81. These results are in accordance with the literature: Maucheline *et al.* (2005) compared different essential oils at 10% dilution and observed the following mean RV: 0.97 for peppermint (*Mentha piperita*), 0.97 for lavender, 0.95 for Tea tree, and 0.9 for eucalyptus.

Prices of different essential oils

In addition to a high repellency value, the price of an essential oil is a major factor to choose candidates for field application strategies. Prices of essential oils fluctuate during the year, depending on origin and harvesting time of plants. Prices given in Table 1 are rough estimations provided by qualiessentials GmbH. The cheapest essential oil in our experiments was grapefruit oil. Lemongrass and *Litsea* oil are also reasonably priced. Cornmint oil is considerably more expensive and lavender oil was by far the most expensive oil in our experiments. Based on the results of the experiments and on the prices of the essential oils, the development of a field application strategy will focus on cornmint oil, lemongrass oil and *Litsea cubeba* oil.

Table 1. Repellency values (± se) of different essential oils tested in the Y-tube-olfactometer containing "flower clusters + filter paper + acetone" and "flower clusters + filter paper +essential oil diluted 1:10 in acetone" and estimation of price per kg of the essential oils.

Essential oil	RV±se	Wilcoxon Test	Tukey HSD-Test	Prices of essential oils
Cornmint	1.00 ± 0.00	*	D	31.50 € / kg
Lemongrass	0.92 ± 0.06	*	CD	17.50 € / kg
Litsea	0.92 ± 0.06	*	CD	18.00 € / kg
Heliosol	0.92 ± 0.04	*	CD	18.00 € / kg
Tea tree	0.89 ± 0.06	*	BCD	32.00 € / kg
Grapefruit	0.86 ± 0.03	*	ABC	14.00 € / kg
Fir needle	0.83 ± 0.06	*	ABCD	28.50 € / kg
Star anise	0.83 ± 0.06	*	ABCD	22.50 € / kg
Lavender	0.81 ± 0.05	*	ABCD	104.00 € / kg
Clove	0.81 ± 0.08	*	ABC	30.00 € / kg
Lemon	0.75 ± 0.08	n.s.	ABC	
Eucalyptus	0.69 ± 0.07	n.s.	ABC	
Wintergreen	0.69 ± 0.11	n.s.	ABC	
Orange	0.67 ± 0.06	n.s.	AB	
Cedarwood	0.64 ± 0.05	n.s.	A	

(Statistical analysis: Wilcoxon signed rank test testing if RV is different from 0.5 with $p < 0.05$; Tukey: Data transformed [arcsin√(x)], Four-way-ANOVA: essential oil: $F_{14,72} = 5.03$, $p < 0.0001$; temperature: $F_{1,72} = 21.57$, $p < 0.0001$; humidity: $F_{1,72} = 0.12$, $p < 0.13$; position of olfactometer arms: $F_{1,72} = 7.73$, $p = 0.007$; Tukey HSD post hoc tests $\alpha = 0.05$, different letters show significant differences).

Acknowledgements

A thousand thanks to Christian Urech who spent the splendid days of spring in the dark room patiently watching beetles speeding through the olfactometer. Many thanks to Brian Baker for language editing the manuscript. Many thanks to qualiessentials GmbH for providing the essential oils. The project was funded by Migros Genossenschaftsbund and supported by Biofarm Genossenschaft.

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Differential rates of attack of oilseed rape genotypes by the pollen beetle: the cues may be in the bud wall

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Abstract: Attraction of the pollen beetle (*Meligethes aeneus*) towards oilseed rape (*Brassica napus*) crops has been well studied over the last 20 years. Volatile compounds emitted by plants, especially isothiocyanates, are the main cues used by adults to locate their host plant. However, little is known about the interaction between the plant and the insect once it arrives on the crop plant. Determining which cues are responsible for the attack once the insect is in contact with its host plant could help to design new crop protection strategies based on resistant (i.e. less attacked) cultivars. We investigated the feasibility of this strategy in the laboratory, by using six oilseed rape genotypes in a no choice feeding experiment. Results clearly showed that a large variation exists in bud attack levels among plant genotypes, with a difference between the two extreme genotypes up to 2.5 times. We further looked for the determinants of the attack, by hypothesizing that the balance between phagostimulants and deterrent compounds in the tissues eaten by the beetles (i.e. bud wall and stamens) could be the origin of the observed variability. For that purpose we analysed compounds present in bud wall and stamens of the different genotypes studied, by using gas and liquid chromatography. Fifty compounds from different biochemical classes (sugars, free amino acids, glucosinolates and flavonoids) were identified and quantified. We found that chemical composition of the bud wall was very well correlated to attack level, showing that this tissue could play an essential role in the interaction between the plant and the insect. Some of the identified compounds, known from the literature to be phagostimulant or deterrent for other beetles, could play a crucial role. Artificial feeding experiments are needed to confirm the role of these compounds in the contrasting levels of attack found.

Key words: oilseed rape, *Meligethes aeneus*, contact cues, phagostimulants, bud wall

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IOBC-WPRS Bulletin Vol. 104, 2014
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The impact of semi-natural habitats on the abundance of pollen beetle adults on winter oilseed rape fields

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Abstract: Pollen beetle dispersal in the field depends on several different factors, such as the phenological stage of the crop, its odour and yellow colour during flowering are especially attractive to pollen beetles. The dispersal of the scent depends on wind direction and pollen beetles' dispersal follows upwind anemotaxis.

This experiment was conducted to investigate the effect of different semi-natural habitats (woody linear, woody areal, herbaceous linear and herbaceous areal) surrounding winter oilseed rape fields on the abundance of the pollen beetle. Beetles were counted from oilseed rape plants using the beating method. The results showed higher number of pollen beetles on fields bordered with herbaceous linear elements than with other studied semi-natural habitat elements.

Key words: *Meligethes*, oilseed rape, semi-natural habitat, dispersal

Introduction

Oilseed rape is the third most widely cultivated crop in Europe (FAO, 2013), the sowing area has also increased rapidly in Estonia. One of the main challenges in its production is the control of insect pests. The most widespread species causing yield losses throughout Europe is the pollen beetle (*Meligethes aeneus* Fab. Coleoptera: Nitidulidae) (Alford *et al*., 2003).

Several aspects of pollen beetle oviposition and feeding preferences, overwintering and crop location have been intensively studied (Free & Williams, 1978; Borg, 1996; Giamoustaris & Mithen, 1996; Alford *et al*., 2003; Ferguson *et al*., 2003; Veromann *et al*., 2006, 2009, 2012, 2013; Williams *et al*., 2007; Williams, 2010; Williams & Cook, 2010), but the influence of landscape elements on the dispersal of the pollen beetle is less studied. Rusch *et al.* (2012) have found that overwintering habitats of the pollen beetle are influenced by the characteristics of the local habitat. Nevertheless, the impact of specific landscape elements such as semi-natural habitats on pollen beetle dispersal remains unknown.

The aim of the study was to determine the impact of different commonly occurring semi-natural habitats to pollen beetles' dispersal in field conditions.

Material and methods

The study was carried out in 2013 on commercial winter oilseed rape fields in Estonia. The effect of four different semi-natural habitats (SNH): woody area (Wa), woody linear (Wl), herbaceous area (Ha) and herbaceous linear (Hl) was studied. To determine SNH as 'areal' or 'linear', the length and width of elements were taken into consideration. The length of a linear element was at least 150 m and the width did not exceed 12.5 m: the measurements of areal elements were at least 60 x 60 meters. Oilseed rape fields were selected based on the surrounding landscape; only fields directly boarded with the mentioned SNH-s were selected. In total, the abundance of the pollen beetle was studied in 12 sample points on oilseed rape fields bordered by 3 Wa, 3 Wl, 3 Ha and 3 Hl.

Pollen beetles were counted from 10 randomly chosen oilseed rape plants 2 meters and 20 meters from the edge of the oilseed rape field bordered by each selected SHN type using the plant beating into a tray method (Cooper & Lane, 1991).

Statistical analyses were carried out using programs Statistica and MS Excel. The effect of semi-natural habitat type was measured using Kruskal-Wallis ANOVA, the differences between SNH-s were found using post-hoc Duncan test.

Results and discussion

The bordering semi-natural habitat had a significant effect on the abundance of the pollen beetle ($H(3, 1200) = 47.93$, $P < 0.0001$). The greatest abundance of pollen beetles was recorded on sites bordered by Hl elements, significantly more than all other tested landscape elements ($P < 0.0001$, Duncan test).

The largest number of beetles was found next to the most simple landscape element, Hl. This result concurs with Thies and Tscharntke (1999), who found the complexity of landscape to reduce the damage caused by pollen beetles. This is probably caused by the wind and plant cover concurrence as pollen beetles fly upwind towards suitable food source (Evans & Allen-Williams, 1994; Moser *et al.*, 2009; Williams *et al.*, 2007) which is located using volatile cues provided by food-plants (Evans & Allen-Williams, 1994; Blight & Smart, 1999; Smart & Blight, 2000; Cook *et al.*, 2002). As non-host plants can mask the host plant odours and reduce pest detection and therefore damage (Root, 1973; Pimentel, 1961) larger and more diverse habitats are more likely to provide more odour cues and therefore confuse pest colonization to the crop. Since the perception of the scent as well as its dispersal depends on wind speed (Finch, 1980; Schoonhoven *et al*., 2005) and several semi-natural landscape elements can act as wind barriers, the landscape composition affects the dispersal of insects. Wind is considered to be the most important parameter affecting the detection of odour plume by insects (Beyaert & Hilker, 2013) and therefore influencing their dispersal. For example, several studies have shown that hedgerows reduce the dispersal of insects (Lewis, 1969; Bowden & Dean, 1977; Fry, 1994; Mauremooto *et al*., 1995). This is also confirmed in the current study as significantly less beetles could locate the suitable food source next to woody landscape elements (Wl and Wa) compared to the element which due to its dimensions could not hide the attractive attributes of oilseed rape from the pollen beetle.

Also the width of the area seems to be a relevant factor, as more pollen beetles were found beside Hl elements compared to Ha. This is likely that the height and composition of the vegetation layer has an effect on the host location of pollen beetles. As mentioned before, they rely on chemical cues provided by plants. Specialist herbivores use plant-specific odour clues to locate suitable host plants (Hare, 2011; Pare & Tumlingson, 1999) but plant odour can change over time and distance (Helming *et al.*, 2004). Therefore the increased distance between the release point of the scent in concurrence with the vegetation composition on that trail decreases the probability to reach the source.

In the current study, the Ha element therefore reduces the likelihood to reach the oilseed rape field by confusing the pollen beetle as Wa and Wl have a hindering impact on the wind flow and therefore prevent the spreading of odour.

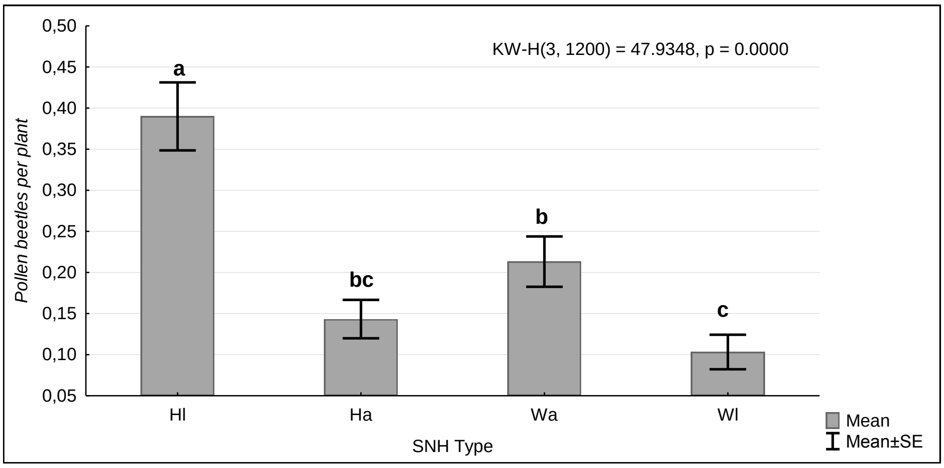

Figure 1. Abundance of pollen beetles on oilseed rape fields boarded by different semi-natural habitats (Hl – herbaceous linear; Ha – herbaceous areal; Wa – woody areal; Wl – woody linear). Different letters indicate significant differences between variables $p < 0.05$; Duncan test.

To conclude, our results demonstrate the importance of commonly occurring landscape elements on the dispersal of the pollen beetle.

Acknowledgements

This study was supported by the project QuESSA that is funded by the European Commission through the Seventh Framework Programme; Estonian Science Foundation grant 8895 and the Estonian Ministry of Science and Education grant SF0170057s09.

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Flight of *Meligethes aeneus* at a range of altitudes

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Abstract: The pollen beetle, *Meligethes aeneus*, is a significant pest of oilseed rape crops and there is considerable research effort focused on developing novel, sustainable methods of integrated control. These insects rely on flight for all dispersal movements and we have investigated their flight patterns using a novel combination of data from suction traps, vertical-looking radar and field counts. Analysis of these preliminary data will help determine the best timing for different control measures within an integrated pest management strategy.

Key words: pollen beetle, oilseed rape, flight, behaviour, altitude

Introduction

Adult *Meligethes aeneus* fly to flowering plants, on emergence from overwintering, to feed on pollen (Free & Williams, 1978). During this early part of the year, there is a temperature threshold for flight in this species. The lowest temperature for a solitary flight was recorded as 10.2 °C (Laska & Kocourek, 1991), however the first gregarious flights (Cooter, 1977; Kenward, 1984) are seen at between 12.3 °C and 15 °C (Tamir *et al.*, 1967; Tulisalo & Tuomo, 1986; Laska & Kocourek, 1991; Sedivy & Kocourek, 1994). Individual beetles fly to flowering oilseed rape crops, firstly winter sown crops (in April/May) where a high intensity of flights was recorded by Sedivy & Vasak (2002) followed by movement to spring sown crops (in June/July) to feed and reproduce. The new generation adults emerge in mid-July and also fly to food sources. Individuals move to feed on other flowering plants once the oilseed rape crops have finished flowering and then finally move to overwintering sites.

Pollen beetle adults are not ground-active as they are rarely caught in pitfall traps, even in oilseed rape fields (Cook & Skellern, unpublished data), therefore, it is assumed that they rely on flight for all dispersal movements. However, all of the movements of individuals described above have been inferred from counts of adults through the year at different sites, not from any direct recording of their flights. Due to their small size, it has proved difficult to track individuals, however some studies have been able to estimate the distances flown. Individuals have been recorded travelling 13.5 km using radioactive tracers (Tamir *et al.*, 1967), but it is likely that they can travel a considerable distance further. Tamir *et al.* (1967) showed that the insects were able to locate and travel to fields of oilseed rape regardless of wind direction indicating that they used self-powered, directed flights rather than being blown by the wind.This is supported by more recent studies (Williams *et al.*, 2007; Evans & Allen-Williams, 1994) which show that adult *M. aeneus* use up-wind anemotaxis to locate oilseed rape plants.

Previous studies have shown that *M. aeneus* is capable of sustained, powered flights towards attractant sources, although the greater distance, high altitude, dispersal movements have not been studied. The experimental work in this paper aimed to investigate this important part of their ecology. This was approached using a novel combination of techniques; field counts, suction traps and vertical-looking radar to provide information on movements both at the ground level and at a range of altitudes.

The aims of this work were:

1. to identify diurnal patterns of flight movements of *M. aeneus*
2. to establish seasonal flight patterns and their use of flight at a range of altitudes throughout their active season.

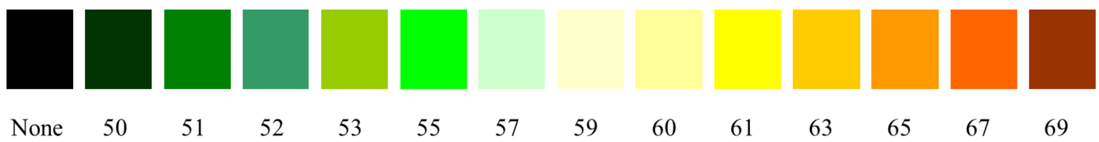
Material and methods

All data were collected at Rothamsted Research, Harpenden, UK from March – August 2001.

Field assessments

Weekly assessments of populations of *M. aeneus* were conducted in five fields of oilseed rape on Rothamsted Farm from March to July. Four 60 m linear transects were sampled in each field; an oilseed rape plant was sampled every 6 m along each transect and a record made of the growth stage (Lancashire *et al.*, 1991) and the number of *M. aeneus* adults present.

The modal value for the growth stage of winter and spring oilseed rape was calculated weekly across all fields and is represented in the results using a colour code (Figure 1).



None - no flower heads; 51-57 green bud; 59 – yellow bud; 60 – first flowers open; 61-63 early flowering; 65- full flower; 67-69 flowering declining

Figure 1. Visual representation of modal growth stage of oilseed rape crops.

Suction traps

The aerial density of *M. aeneus* was measured using the Rothamsted Insect Survey suction traps http://www.rothamsted.ac.uk/insect-survey/. The density was measured at two heights; 1.5 m and 12 m. Both traps were run continuously and the samples collected daily. The samples were stored in 70% ethanol with glycerol in sealed glass vials. Samples were taken from March to the end of August 2001 and the number of *M. aeneus* recorded. The daily catch (restricted to 12 hours i.e. daytime) of insects was converted to aerial density in 10^3 m^3 air sampled.

A second 12 m suction trap was operated from 19th May to 23rd August to investigate the diurnal flight activity of *M. aeneus*. Insect samples from four time periods were collected separately using a timed bottle changer attached to the suction trap.

Vertical-looking radar

Vertical-looking radar (VLR) is a recently developed technique specifically intended to routinely monitor the flight behaviour of migrant insects (Chapman *et al.*, 2003). The VLR is sited on the top of a building at Rothamsted Research and overflying insects modulate the radar signal in a way that is related to their speed and direction of movement, their orientation, size and shape.

Meligethes aeneus weigh between 1 and 2 mg, the radar can only detect this size of insect at the lowest sampling band of 150-195 m above ground level. The amplitudes of any signals captured within that range gate were recorded for a 5-minute period every 15 minutes, 24 hours a day. The aerial densities of daytime records of correct-sized targets were summed and corrected to provide density in 10^3 m^3 air to be comparable with the suction trap density data.

In order to establish exactly which insect species were flying at this altitude, flying insects were collected using an aerial netting technique (Chapman *et al.*, 2002); a sampling net was suspended from a balloon flying at 180-200 m (the same height as the records from the radar) at Cardington airfield, UK. The insect samples from several days' netting were examined for specimens of *M. aeneus*.

Results and discussion

Diurnal flight activity: from 12.2 m suction trap data

The diurnal activity of *M. aeneus* was studied using a 12.2 m suction trap collecting dawn, day, dusk and night samples in 2001. The total numbers of beetles caught throughout the four-month sampling period are shown in Table 1.

Table 1. Diurnal activity of *M. aeneus*; total numbers caught in different time periods at 12.2 m in 2001 and standardised to number per sampling hour.

Sampling period	**Total *M. aeneus* caught per sampling hour**
Dawn (06:00 – 08:00)	1.5
Day (08:00 – 18:00)	21.1
Dusk (18:00 – 20:00)	9
Night (20:00 – 06:00)	0.5

These results indicate that flight occurs predominantly during the daytime in this species, which concurs with the findings of Lewis & Taylor (1965), who showed that the peak flight time in this species was 12.44 GMT.

Seasonal patterns of flight activity

This study has used a novel combination of techniques; field counts, suction trap catches and VLR data. The data provides evidence, for the first time, that *M. aeneus* utilises flight at a range of altitudes, up to at least 200 m.

- The total number of *M. aeneus* caught throughout the season in the suction traps was 136 in the 1.5 m trap and 237 in the 12 m trap
- There were 1916 records of 1-2 mg insects from the VLR data; a proportion of which were assumed to be *M. aeneus.*
- Aerial netting samples from 1999 collected a total of 15 *M. aeneus* in 7 of the 9 sampling days and in 2000, a total of 41 *M. aeneus* were caught during 8 of the 11 sampling days.

The density of *M. aeneus* at 1.5 m, 12 m and 200 m was calculated weekly from March to August in 2001 (Figure 2B). The highest density of beetles occurred at 1.5 m whereas the 12 m and 200 m densities are approximately one order of magnitude smaller. The mean number of *M. aeneus* beetles per oilseed rape plant from all the fields sampled was calculated weekly and plotted for 2001 (Figure 2A) for both winter and spring oilseed rape crops.

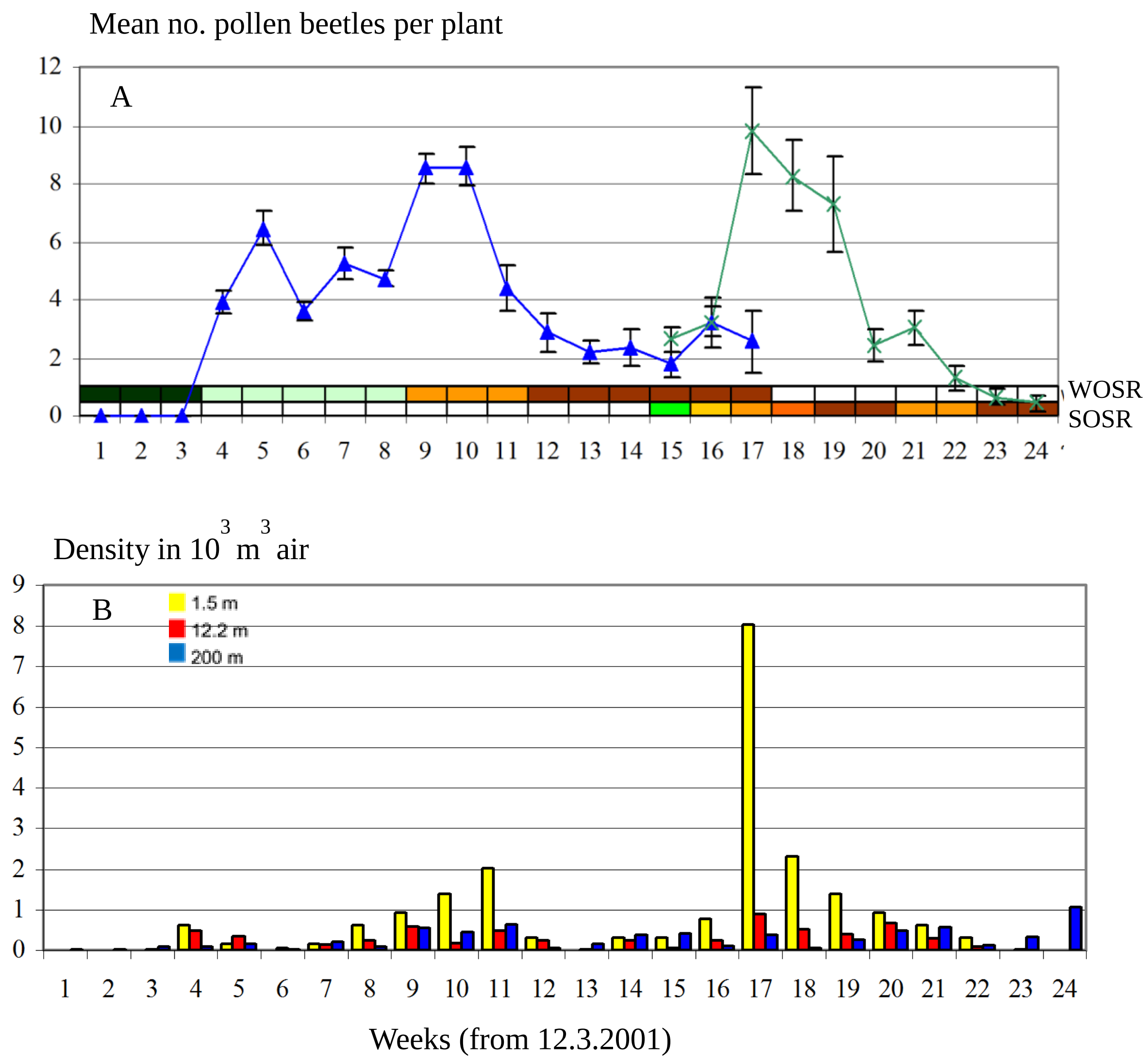


Figure 2A. Mean (± SE) number of *M. aeneus* per plant in winter (in blue) and spring (in green) oilseed rape crops. Coloured bar along the bottom shows the crop phenology of winter (WOSR) and spring rape (SOSR) (refer to Figure 1).
Figure 2B. Suction trap and Vertical Looking Radar weekly densities of *M. aeneus* per 10^3 m^3 air in 2001.

Conclusions

This study has shown, for the first time, an altitudinal profile of *M. aeneus* throughout their active season. It has provided evidence that this species uses high altitude flights (up to 200 m) as well as low altitude flights. It has also confirmed that there is a strong tendency for flight during the day, which might have resulted from a temperature threshold requirement for flight and/or a strong reliance on visual cues during flight at altitude that are unavailable at night.

The novel combination of techniques used in this study has provided a method for long-term monitoring of the population movements of this pest, and the modelling of several years' worth of data could potentially yield specific predictors of immigration to oilseed rape crops.

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Developing and integrated pest management strategy for pollen beetles in oilseed rape: Results from the Defra SA-LINK project LK09108

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Abstract: We have developed an integrated pest management strategy (IPM) for pollen beetles in winter oilseed rape (OSR) based on risk assessment, monitoring and alternative crop management that can be used as a framework by growers and crop consultants to manage pollen beetles with reduced insecticide inputs - and the confidence to do so. This will prolong insecticide life by reducing selection for resistance, reduce environmental impacts and contribute towards the sustainability and profitability of OSR in the UK. One of the major limitations to the use of action thresholds is that proper monitoring of the populations is time consuming and has to be conducted over a prolonged period. To encourage and facilitate their use, we tested and developed tools to improve risk assessment and monitoring. We conducted a pollen beetle monitoring study over 4 years in 178 OSR crops across the UK. Pollen beetles were sampled using sticky traps and plant sampling along transects in the crop. The data were used to help test a decision support system (DSS) for pollen beetles and to develop a monitoring trap. proPlant Expert is a DSS available in mainland Europe that uses a model of pollen beetle immigration and local meteorological data to forecast the start and end of pollen beetle immigration into the crop and main risk periods and advises when to monitor. We tested the model under UK conditions using data from our study and compared monitoring advice with the current advice system on the CropMonitor website (advises monitoring when the crop is at green-yellow bud stage and temperature >15 °C). Both performed reassuringly well in prompting monitoring that would detect breaches of spray thresholds. However there were considerable reductions provided by proPlant in the need for consultation of the system (30%) and advised monitoring days (34-53%) in comparison with current advice. Use of the proPlant DSS could therefore focus monitoring effort to when it is most needed. It could also help to reduce unnecessary sprays in cases where beetle numbers are approaching threshold but consultation of the system returns a poor immigration risk forecast or an immigration complete result. The proPlant tool is now freely available to growers and crop consultants in the UK via the Bayer CropScience website. A monitoring trap for pollen beetles would help to more easily and accurately identify when spray thresholds have been breached than monitoring plants in the crop. We developed a baited monitoring trap for pollen beetles which will be commercially available from Oecos. The trap comprises a yellow sticky card mounted at 45°, baited with phenylacetaldehyde, a floral volatile produced naturally by several plant species. Unfortunately using data from our study we were unable to calibrate the trap catch to a given action threshold expressed as the number of beetles per plant using a simple linear relationship. However, the monitoring trap still has value for risk assessment, especially if used together with DSS. We tested the potential of turnip rape (TR) trap crops, planted as borders to the main OSR crop to reduce pollen beetle numbers in a field scale experiment conducted over three years on two sites. We found evidence that the strategy worked well in some years, but not others. This tactic is probably practically and economically worthwhile only for organic growers.

Key words: Pollen beetle, *Meligethes aeneus*, petal colour, visual cues, photoreceptors

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Results of a small survey amongst farmers and advisers in the UK on their evaluation of the proPlant pollen beetle migration tool and its influence on their practice

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Abstract: One of the major impediments to the use of action thresholds for pollen beetle control in oilseed rape has been that proper field monitoring of beetle populations is time consuming and is potentially required over a prolonged period. proPlant Expert is a decision support system (DSS) that uses phenological models parameterised by local meteorological data to forecast pest risks. It is able to forecast the start and end of pollen beetle immigration into the crop and to advise of the main risk periods and when to monitor. The proPlant expert.map pollen beetle migration forecasting tool was made publicly available in the UK on the Bayer CropScience website in spring 2012, encouraged by the success of trials by Rothamsted. Three forecasting maps were provided each day with a traffic light warning system indicating: (1) the risk of migration starting (2) the risk of new migration (3) percent completion of migration. We conducted a small impact survey in spring 2012 to obtain feedback from users of the forecasting tool to support future improvements and delivery of the tool and thereby to encourage uptake. The survey consisted of a one page multiple-choice questionnaire with 17 questions grouped under five headings.

Eighteen farmers and agronomists responded to the call for survey participants. Of these, 10 completed the survey. In this small survey there was overwhelmingly positive feedback. There was clear endorsement of the manner in which the pollen beetle risk forecasting tool was presented and explained. Most users found the tool informative and all found it helpful. Feedback indicated that users were making intelligent use of proPlant expert.map in the context of their experience in the field, as intended. Respondents found that the forecasts corresponded with events in the field and reported that the tool increased their confidence in decision-making, giving them peace of mind. Moreover, using proPlant expert.map reduced eight out of ten users' estimation of pollen beetle risk and seven believed they had used fewer sprays for pollen beetle control as a result. Eight out of the ten respondents said they would certainly recommend proPlant expert.map to a friend.

Key words: Pollen beetle, *Meligethes aeneus*, decision support systems (DSS), proPlant, Bayer Pollen Beetle Predictor

Introduction

A major constraint to the use of action thresholds for control of pollen beetle (*Meligethes aeneus*) in oilseed rape is that proper field monitoring of beetle populations is time consuming and is needed over a prolonged period. proPlant Expert (http://www.proplantexpert.com) has become widely used in Europe for pest management in winter oilseed rape crops (Johnen et al., 2010; Johnen & von Richthofen, 2013; Newe *et al.*, 2003). It is a web-based decision support system (DSS) that uses phenological models driven by weather data (air temperature, rainfall, sunshine and wind speed) automatically downloaded from local meteorological stations. It provides local three-day forecasts of pollen beetle migration risk and indicates days when crop monitoring is needed.

To support and facilitate the use of action thresholds for pollen beetle control, we tested the proPlant expert.map tool under UK conditions as part of the 2008-2012 UK Defra-sustainable arable LINK project LK09108 'Developing an integrated pest management strategy for pollen beetles in winter oilseed rape', which was co-funded by the HGCA (Cook *et al.*, 2013). We found that the model, originally designed using data from mainland Europe, predicted pollen beetle immigration extremely well in the UK's maritime climate (Ferguson *et al.*, 2013). Encouraged by this, in spring 2012 the proPlant expert.map pollen beetle migration forecasting tool was made publicly available in the UK on the Bayer CropScience website (www.bayercropscience.co.uk/) as the 'Bayer Pollen Beetle Predictor'.

The aim of the work reported here was to perform a small impact assessment to gain user feedback in the first year of release of the proPlant expert.map pollen beetle migration forecasting tool as the 'Bayer Pollen Beetle Predictor', and to evaluate how using the proPlant system in 2012 influenced monitoring and spraying practices.

Material and methods

Recruitment of farmers and agronomists to the study

In March 2012, a request for people to take part in a feedback survey of proPlant expert.map was made publicly available as the 'Bayer Pollen Beetle Predictor'on the Bayer website was advertised through the farming press, HGCA, Rothamsted's Twitter account and by direct approach to more than 100 farmers.

Version of the proPlant model tested

The study tested the response of farmers and agronomists to proPlant expert.map in spring 2012. This tool provided three forecasting maps for each day:

a. Start migration – indicates whether pollen beetles have started to migrate this season
b. New migration – indicates whether further migration is expected today or in the next 2 days
c. Percent migration – indicates percent completion of pollen beetle migration to date.

The forecast for different locations in Great Britain were indicated by traffic-light coloured-coded squares (Figure 1) which could be interrogated for location-specific information by holding the cursor over the square. Instructions for use of the maps were provided on the Bayer CropScience website and were copied to the farmers and agronomists who responded to the call for survey participants.

Survey form

The survey consisted of a one page multiple-choice questionnaire with 17 questions grouped under five headings (Figure 2):

A. Ease of understanding and interpretation of proPlant expert.map for pollen beetles
B. Your use of proPlant expert.map
C. Influence on your management of pollen beetles
D. Your over-all evaluation of proPlant expert.map
E. Where did you hear about proPlant expert.map forecasting tool?

Each question was answered by placing a cross in one of three or four tick-boxes. Respondents were permitted to use more then one tick-box for questions under headings B and E, if appropriate. Respondents were invited to add any further comments or clarification on an attached blank sheet. The survey was designed to completed electronically and returned by email or to be printed and completed by hand.

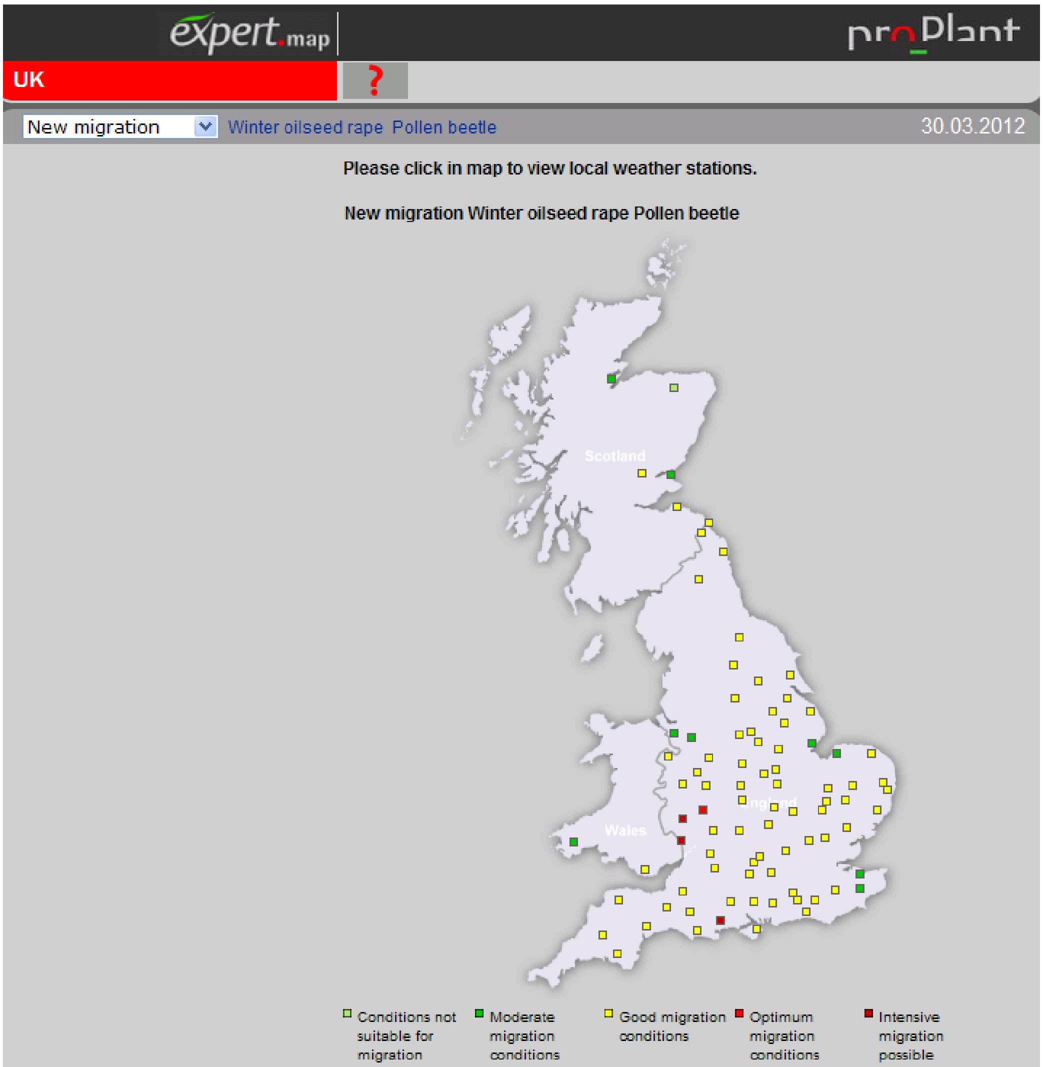

Figure 1. Example of a proPlant expert map forecast for Great Britain from 30/03/2012.

Results and discussion

Survey response rate

Eighteen farmers and agronomists responded to the call for survey participants. Of these, 10 completed the survey, giving a response rate of 56% (60% of whom were advisors, 40% were growers).

Summary of survey responses

Survey responses are summarised as totals in each tick-box in Figure 2. The total number of ticks for each question was 10, corresponding to the number of respondents, except for questions in sections B & E where respondents were invited to tick more than one box if applicable.

proPlant expert.map pollen beetle management tool user feed-back survey 2012

IN CONFIDENCE. Summary results will be published in grouped, anonymous form.

To answer the questions, please put X in the box that best represents your response.

Section A: Ease of understanding and interpretation proPlant expert.map for pollen beetle

1. Was the purpose of proPlant expert map clear?

very clear	fairly clear	not very clear	unclear
6	4		

2. Were the instructions on the Bayer CropScience website for use of the pollen beetle forecast maps clear? To remind you, these instructions are appended to the covering email.

very clear	fairly clear	not very clear	unclear
6	4		

3. Was the proPlant expert.map interface user-friendly and easily understood?

very clear	fairly clear	not very clear	unclear
5	5		

4. Was the guidance on interpreting maps clear?

very clear	fairly clear	not very clear	unclear
5	5		

5. Was it clear that new breaches of spray thresholds are unlikely when migration is forecasted to be complete?

very clear	fairly clear	not very clear	unclear
5	3	2	

6. Scenario: I have 10 beetles per plant now and I'm concerned that beetles might very soon exceed threshold. Dare I risk waiting if I have a spray window today? Was it clear that proPlant can help with this decision?

very clear	fairly clear	not very clear	unclear
3	5	2	

Section B: Your use of proPlant expert.map

7. How often did you consult proPlant expert.map?

daily	every 2-3 days	weekly	less often
2	6	3	

8. Which forecast did you consult?

Start migration	New migration	Percent migration
6	10	6

Section C: Influence on your management of pollen beetles

9. Did using proPlant expert.map influence the amount of pollen beetle monitoring you did?

Yes	No	I was monitoring regularly for Rothamsted
9	1	

10. If you answered 'yes', did you monitor

more often	less often	about the same
3	6	

11. Did using proPlant expert.map influence your estimation of the risk of pollen beetle damage?

Yes, more risk	Yes, less risk	No
	8	2

12. Do you think that using proPlant changed your pollen beetle management this year?

Yes, more sprays	Yes, fewer sprays	No
	7	3

13. Do you expect that using proPlant would affect your management of pollen beetles over the long term?

Yes, more sprays	Yes, fewer sprays	No	Don't know
	8		2

Section D: Your over-all evaluation of proPlant expert.map

14. Did you find proPlant expert.map informative?

yes, very	yes	not very	no
4	5	1	

15. Did you find proPlant expert.map helpful?

yes, very	yes	not very	no
4	6		

16. Would you recommend proPlant expert.map to a friend?

yes, certainly	probably	probably not	no
8	1	1	

Section E: 17. Where did you hear about proPlant expert.map forecasting tool? X in all boxes that apply

Rothamsted	Bayer website	Farming press	other *
7	5		2

* please specify other: Via Bayer representatives: 2

If you would like to add any further comment or clarification, please use the blank sheet below

Many thanks! Please save this & return to Dr. Sam Cook, Rothamsted sam.cook@rothamsted.ac.uk

Part of a project led by Dr Sam Cook of Rothamsted Research: LK0108 - additional work: Improving decision support and risk assessment in IPM of pollen beetle in oilseed rape. It is funded by the HSE Chemicals Regulation Directorate.

Figure 2. proPlant expert.map pollen beetle management tool user feed-back survey form 2012, with response totals. Note that respondents were able to tick more than one box in sections B & E.

A. Ease of understanding and interpretation of proPlant expert.map for pollen beetles

Overall the responses of participants to the questions in this section represented a strong endorsement of the manner in which the pollen beetle risk forecasting tool proPlant expert.map was presented and explained and of the user interface. Respondents were given a choice of four tick boxes marked 'very clear', 'fairly clear', 'not very clear' and 'unclear' for their responses to each of six questions. Only four out of sixty responses were allocated outside the 'very clear' or 'fairly clear' categories and these were scored 'not very clear' (Figure 2). All ten respondents ticked 'very clear' of 'fairly clear' in response to the following four questions: Was the purpose of proPlant expert.map clear? Were the instructions for its use on the Bayer CropScience website clear? Was the user interface user-friendly and easily understood? Was the guidance on interpreting the maps clear?

Respondents were not quite so clear about the context-specific interpretation of proPlant expert.map that was explored in questions five and six. When migration was forecasted to be complete, eight respondents found that it was very clear or fairly clear that new breaches of spray thresholds were unlikely, but two found that it was not very clear. Question six presented respondents with the scenario that the number of pollen beetles per plant on the crop was approaching the control threshold and that conditions were right for spray application that day. Eight respondents thought that it was very clear or fairly clear that proPlant expert.map could help with the decision as to whether to delay control but two found that it was not very clear. These reservations on the interpretation of proPlant expert.map are consistent with a respondent's suggestion that more training would be helpful (see *Respondents comments* below).

B. Your use of proPlant expert.map

Six out of ten respondents consulted proPlant expert.map every 2-3 days (Figure 2). One respondent consulted the tool daily while another made a more sophisticated use of the tool, consulting it every 2-3 days and increasing to daily consultations "when the data suggested a changing situation". Three respondents consulted proPlant expert.map only weekly, which may be considered less than optimal. Despite this, only one of these three felt that use of the tool had not influenced their management of pollen beetles (see section C below).

All ten respondents consulted the 'new migration' forecast but only six of these also consulted the 'percent migration' forecast. This suggests that the potential value of percent migration estimates in determining whether significant new migrations are likely is not fully understood. This is consistent with the lack of clarity expressed by some users about the usefulness of proPlant expert.map in the context-specific scenarios in questions five and six in section A of the survey.

Guidance for users is needed as to the value and potential use of estimates of percent migration if the potential for this tool to reduce unnecessary insecticide applications is to be realised. This guidance should make clear that the new migration forecast indicates only that conditions are suitable for migration. The percent migration forecast gives some information about the potential scale of any new migration, larger new migrations being less likely when percent migration is already close to 100%. This information should be accompanied by a warning that none of the forecasts provide information on the actual number of migrating pollen beetles, which is influenced by factors outside the paramenters of the proPlant model, such as previous reproductive and overwintering success. Information provided by proPlant expert.map is not a stand-alone tool but is of value in prompting appropriate monitoiring. It's value is enriched by the information provided by monitoring.

C. Influence on your management of pollen beetles

Nine out of ten respondents said that using proPlant expert.map influenced the amount of pollen beetle monitoring they did, three monitoring more often and six less often than they otherwise would have done (Figure 2). The full implications of this are hard to determine as no question on their previous practice was included. However, the fact that the practice of the large majority was changed, suggests a strong engagement with the information provided by the tool.

There was remarkably strong evidence that using proPlant expert.map reduced users' estimation of pollen beetle risk and of the number of sprays they used for pollen beetle control. Eight out of ten respondents said that using proPlant expert.map reduced their estimation of the risk and seven said they thought they had used fewer sprays as a result of using it, none found it had increased their estimation of risk or their spray use. Encouragingly, eight respondents expected that using proPlant over the long term would reduce the number of sprays they used for managing pollen beetles.

D. Your over-all evaluation of proPlant expert.map

Responses in this section represented a clear endorsement of the perceived value of proPlant expert.map. Nine out of ten users found the tool informative or very informative and all ten found it helpful or very helpful (Figure 2). Remarkably, eight out of the ten respondents said they would certainly recommend proPlant expert.map to a friend, one said they probably would and only one said they probably would not.

E. Where did you hear about proPlant expert.map forecasting tool?

Seven respondents had heard of the proPlant expert.map forecasting tool through Rothamsted and seven through the Bayer CropScience website or from Bayer CropScience representatives. This suggests that personal contact was particularly effective in recruiting respondents. Although the response rate was high amongst those who volunteered to take part in the survey, they represented a small sample of the numbers invited to take part. Caution should therefore be exercised in generalising the encouraging inferences of this survey to all farmers and agronomists. We recommend that a wider survey is conducted and could be integrated into a training programme designed to encourage uptake.

Respondents' comments

Six respondents chose to add further comments on their evaluation of proPlant expert map and on the conduct of this survey. Three of these also gave feed-back by email. All feed-back was constructive and the comments broadly fell into three categories: positive feedback (the majority), negative feedback, and suggestions for future improvement and delivery of the tool.

Positive feedback

Five out of the six respondents who added comments gave positive feedback. Some of it was of a general nature, e.g.: "Found it useful so far"; "The concept is spot on ….. I found it very useful"; "I am very impressed with the data on the maps and how it corresponds with what I am seeing in the field". Other comments indicated how using the tool had influenced their pollen beetle management practice e.g.: "By using the website I had the confidence to not worry that we would have to go back again with another spray"; "I found it useful to monitor the migration levels and really used it to confirm the decisions I had made were correct. ….. I visited the site every 2-3 days but when the data suggested a changing situation I did check on a daily basis. I would anticipate using the tool next year"; "ProPlant took the pressure off the need for daily crop inspections and benefited us in checking beetle numbers as new migrations were forecasted. This gave us greater peace of mind and reduced the amount of insecticide used"; "I was inclined to extrapolate the information from the tool in combination with field observation to make treatment decisions".

This feedback indicates that users were making intelligent use of proPlant expert.map in the context of their experience in the field, as intended. It is particularly encouraging that respondents found that the forecasts corresponded with their experience in the field and that the tool increased their confidence in decision-making, gave them peace of mind and reduced their use of insecticide.

Negative feedback

Two respondents identified problems related to the weather data used by proPlant expert.map: "some hiccups regarding temperature data of certain sites not seemingly being correct", "You have no weather stations in Lancashire". All weather forecasts carry a degree of uncertainty but both of these problems might perhaps be ameliorated by the use of more sources of real meteorological data and/or by the provison of a more dense and comprehensive network of locations for which weather data and pollen beetle migration is modelled.

One respondent said "[I] found it took a few visits [to the website] to fully understand the best way to use it". This could be resolved by the provision of more training materials or training days (see also suggestions for improvement below).

Suggestions for future improvement

There were two main suggestions for impovement, firstly that a more comprehensive network of locations should be modelled by proPlant expert.map and secondly that "more training would be useful".

Conclusions

In this small survey there was overwhelmingly positive feedback from ten users of the version of proPlant expert.map provided on the Bayer CropScience public website in spring 2012 (the Bayer Pollen Beetle Predictor). There was clear endorsement of the manner in which the pollen beetle risk forecasting tool was presented and explained. Most users found the tool informative and all found it helpful or very helpful. Feedback indicated that users were making intelligent use of proPlant expert.map in the context of their experience in the field, as intended. Respondents found that the forecasts corresponded with events in the field and reported that the tool increased their confidence in decision-making, giving them peace of mind. Moreover, using proPlant expert.map reduced eight out of ten users' estimation of pollen beetle risk and seven believed they had used fewer sprays for pollen beetle control as a result. Eight out of the ten respondents said they would certainly recommend proPlant expert.map to a friend.

There was evidence that the proPlant map of % migration was under-utilised, only six out of the respondents having used it, compared to all ten who used the new migration map. This is consistent with the lack of clear understanding expressed by some users about the use of proPlant expert.map in context-specific scenarios. One user called for more training and this may be necessary if the full potential for proPlant expert.map to reduce unnecessary insecticide applications is to be realised.

We recommend that a wider survey is conducted to confirm these results with a bigger sample of users. Such a survey could be integrated with a training programme designed to encourage uptake and to explain proPlant's use in different scenarios, particularly the value of percent migration forecasts. Consideration should also be given to establishing a more comprehensive network of UK locations for modelling by proPlant expert.map.

Acknowledgements

We thank all the growers and advisors who participated in this study and the UK Agriculture & Horticulture Development Board's Home Grown Cereals Authority (AHDB-HGCA), Farming Online, Farmers Weekly, LEAF (Linking Environment And Farming) and Rothamsted Research Association for helping to promote the survey to potential respondents. This work was funded under the Defra Sustainable Arable LINK programme LK09108 by the UK Department for Environment, Food and Rural Affairs and the UK Health and Safety Executive (Chemicals Regulation Directorate. Rothamsted Research is a national institute of bioscience strategically funded by the UK Biotechnology and Biological Sciences Research Council (BBSRC).

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Pathology papers

Integrated Control in Oilseed Crops
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Global warming and oilseed rape pathogens: potential impacts and adaptation strategies in Northern Germany

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Abstract: Within the research framework "KLIFF – climate impact and adaptation research" potential effects of increasing air and soil temperatures on the life cycle of economically important oilseed rape pathogens in Lower Saxony were studied theoretically and experimentally. In climate chamber and field experiments utilizing a soil warming facility, air and soil warming treatments reflected current climate change scenarios in Lower Saxony for the periods 2001-2030 and 2071-2100 as projected by the regional climate model REMO. Two-year investigations included (1) Phoma leaf spot development in autumn as well as subsequent stem canker development in spring (field only), (2) apothecia production of *Sclerotinia sclerotiorum* in spring and (3) the infection of winter oilseed rape with *Verticillium longisporum*.

Climate chamber and field results were compared on a thermal time scale by calculating degree-days (dd) from day of sowing and March 1st until sampling. Regression analysis showed that plant growth in spring responded almost linearly to increasing thermal time, whereas colonization of plant tissue by *V. longisporum* showed an exponential increase when exceeding 1300-1500 dd and reaching plant growth stage BBCH 74/75, potentially leading to higher inoculum densities after harvest and an increased economic importance of this pathogen under future warming. Sclerotia germination of *S. sclerotiorum* reached its maximum at 600-900 dd. Hence, warming may lead to earlier apothecia production and an advance of the infection window, whereas the future importance of the pathogen may remain constant. Severity of phoma crown canker increased linearly with increasing thermal time, but showed also large variation in response to the warming treatments, suggesting that factors such as canopy microclimate in autumn or leaf shedding over winter may play a bigger role for infection and disease severity than higher soil temperatures. Potential direct and indirect adaptation strategies for farmers to encounter these changes, based on experimental and additional modelling results, were presented.

Key words: climate change, *Sclerotinia sclerotiorum*, *Leptosphaeria maculans*, *Verticillium longisporum*, soil warming, degree days

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IOBC-WPRS Bulletin Vol. 104, 2014
pp. 109-114

Field inoculations of winter oilseed rape

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Abstract: The aim of our work was to increase the explanatory power of field tests on the health of oilseed rape. We worked with sclerotia of the pathogen *Sclerotinia sclerotiorum* and conidiospores of *Phoma lingam*. Pathogen isolates were obtained by collecting tissues of infected plants and isolating the pathogen from infected tissue. Sclerotia were applied to the soil at concentration of 1.5 sclerotia per 1 m^2 when sowing oilseed rape. Conidiospores were applied by foliar spray at concentration 20×10^9 per 1 ha in autumn and in spring. Monitoring was carried out on three varieties of winter oilseed rape with different resistance level to monitor the pathogens. Field trials were established in years 2011/2012 and 2012/2013 at two locations in the Czech Republic – in Opava and Šumperk. The results confirmed the effect of year, locations and infection pressure of pathogens. The tests revealed different reactions of the different varieties with respect to artificial inoculation. The experiments revealed different effects of the treatments. Whereas all varieties were more frequently diseased after inoculation with *S. sclerotiorum*, phoma inoculations displayed higher disease incidences only on cultivars Asgard and NK Morse but had no significant effect on cultivar Da Vinci.

Key words: oilseed rape, field inoculation, *Sclerotinia sclerotiorum*, *Phoma lingam*

Introduction

Oilseed rape is the second most important oilseed crop in the world with total production amounting to about 55 million tons of seed. Its biggest producer is the European Union and after that China (Baranyk *et al.*, 2010). In the Czech Republic mostly winter form of oilseed rape is grown. Since 2007 the planting area has increased from 300 to 400 thousand hectares. The most important diseases of oilseed rape, blackleg of crucifers and white rot of oilseed rape, are both significant factors in choosing whether to plant oilseed rape. Blackleg is caused by two pathogens *Leptosphaeria maculans* (Desm.) Ces. et de Not. and *Leptosphaera biglobosa* sp. Nov. Shoemaker & Brun. Both pathogen´s anamorph is *Phoma lingam* (Tode ex Fr.) Desm. (Rimmer & van den Berg, 2007). The natural source of infection are postharvest crop residues and seeds. White rot of oilseed rape is caused by the polyphagous pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary. The disease is widespread across the whole of the Czech Republic and its importance has recently been reviewed (Plachká & Poslušná, 2013). The natural source of infection are sclerotia in the soil and in the postharvest crop residues. The infestation level of these diseases in the Czech Republic strongly depends on local weather conditions (Plachká *et al.*, 2012).

The importance of these fungal diseases means that evaluation of resistance against these diseases forms an important part of the registration procedures with regard to crop varieties. The resistance level against blackleg of crucifers, white rot of oilseed rape and *Alternaria* leaf spot belongs to the important agronomical signatures of oilseed rape varieties (Zehnálek, 2011) and also is of interest to producers of pesticides. In the registration procedure, a lot of importance is given to the fungicides applied to oilseed rape. For these purposes it is important to create favorable conditions for target testing of resistance level of oilseed rape genotypes and the efficacy of fungicides within breeding and registration procedures for varieties. One way to enhance this is to artificially increase the infection pressure of these pathogens under field conditions. In order to obtain data with strong explanatory power we inoculated oilseed rape with *Phoma lingam* conidiospores and sclerotia of *S. sclerotiorum* (Plachká *et al*., 2012).

Material and methods

Isolates

Phoma lingam-isolate OL2000. Isolate classification: *Leptosphaeria biglobosa*. Inoculum concentration was 20×10^9 per 1 ha (spray volume 300 l/ha). Application method: Foliar application in autumn at growth stage BBCH 13-16, in spring at growth stage BBCH 30-33.
Sclerotinia sclerotiorum-Isolate OP2011 and CH2008. Inoculum concentration 1.5 sclerotia per 1 m^2. Application method: Buried in the soil with seed during sowing. Density of sclerotia was determined based on the information of Hoffmann & Schmutterer (1999). They described as a prerequisite of high oilseed rape infestation the formation of three or more apothecia per square meter. We assumed the formation of one or more apothecia from a single sclerotium.

Varieties

Three standard varieties of winter oilseed rape were used: Asgard (Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, DE), Da Vinci (SW Seed Hadmersleben GmbH, DE) and NK Morse (Syngenta Seeds S.A.S., FR). The varieties were chosen to have different, known resistance level to the pathogens being monitored.

Field tests

Field tests were established as small-plot experiments (size of plot 10 m^2). Each variety was established in 4 treatments and in 3 replicates. Trials were established at two sites; Opava and Šumperk.

Trial schedule/treatments:

T1-C Untreated control
T2-S Sclerotia of *Sclerotinia sclerotiorum* – mixed with rape seed when sown
T3-P1 Foliar application of *Phoma lingam* inoculum – in autumn
T4-P2 Foliar application of *Phoma lingam* inoculum – in autumn and spring

Field evaluations were carried out according to the EPPO standard PP 1/78 (3) root, stem foliar and pod diseases of rape. The incidence level of monitored diseases (blackleg, white rot) was evaluated before harvest at growth stage BBCH 83-87.

Description of locations

The locality Opava Kylešovice (Opava region, Silesia) belongs to a predominantly sugar beet cropping area at an altitude of 256 m. The long-term annual average temperature is 8.23 °C and the long-term annual precipitation level is 592.6 mm. The locality Rapotín (Šumperk region, North Moravia) belongs to a predominantly potato cropping area at an altitude of 325 m. The long-term annual average temperature is 7.27 °C and the long-term annual precipitation level is 702.2 mm.

Results and discussion

Sclerotinia sclerotiorum

The natural incidence of white rot of oilseed rape in 2012 in Opava was low and ranged from 0 to 1%. In this season, artificial inoculation with sclerotia did not show increased disease incidence levels. In 2013 the natural disease incidence was evaluated as medium. The influence of artificial inoculation was observed only on variety Da Vinci, where the incidence level increased by 6%. Varieties Asgard and NK Morse were less infected than the non-inoculated control.

The natural incidence of white rot of oilseed rape in Šumperk in 2012 and 2013 was evaluated at low level and ranged between 6 to 15% of evaluated plants. In 2012 the incidence level increased after inoculation by sensitive variety Asgard by about 6%, on variety NK Morse by 7% and on variety Da Vinci by 1%, respectively. In 2013 the disease incidence level increased after inoculation on variety Asgard by 6% again, on variety NK Morce by 9% and on variety Da Vinci by 5%, respectively. Results are given in Table 1.

Table 1. Disease incidence (%) of white rot on different cultivars of oilseed rape in monitored years and localities of the untreated control (T1-C), after artificial inoculation with sclerotia of *S. sclerotiorum* (T2-S) and foliar application of *Phoma lingam* inoculum in autumn (T3-P1) and in autumn and spring (T4).

Year	Location	Opava				Šumperk			
		Treatment				Treatment			
	Variety	T1-C	T2-S	T3-P1	T4-P2	T1-C	T2-S	T3-P1	T4-P2
2012	Asgard	0	1	0	0	15	21	10	12
	Da Vinci	0	0	0	0	10	11	11	11
	NK Morse	1	0	0	0	6	13	5	7
2013	Asgard	31	29	26	30	7	13	11	10
	Da Vinci	20	26	19	22	12	17	7	7
	NK Morse	20	16	19	14	7	16	7	8

In general the response of tested oilseed rape varieties with artificial inoculation with sclerotia of *S. sclerotiorum* was equal. Comparing average results of individual years and localities the difference between incidence levels ranged between 2 to 3%. Results are given in Figure 1.

The results showed that the response of oilseed rape varieties on the artificial inoculation was influenced by the white rot incidence level in individual year in selected localities and by the resistance level of the varieties of oilseed rape. In the case of medium infestation year, the susceptible variety responded more than resistant ones. With low infestation levels, all varieties responded equally with increased infestation level with respect to artificial inoculum.

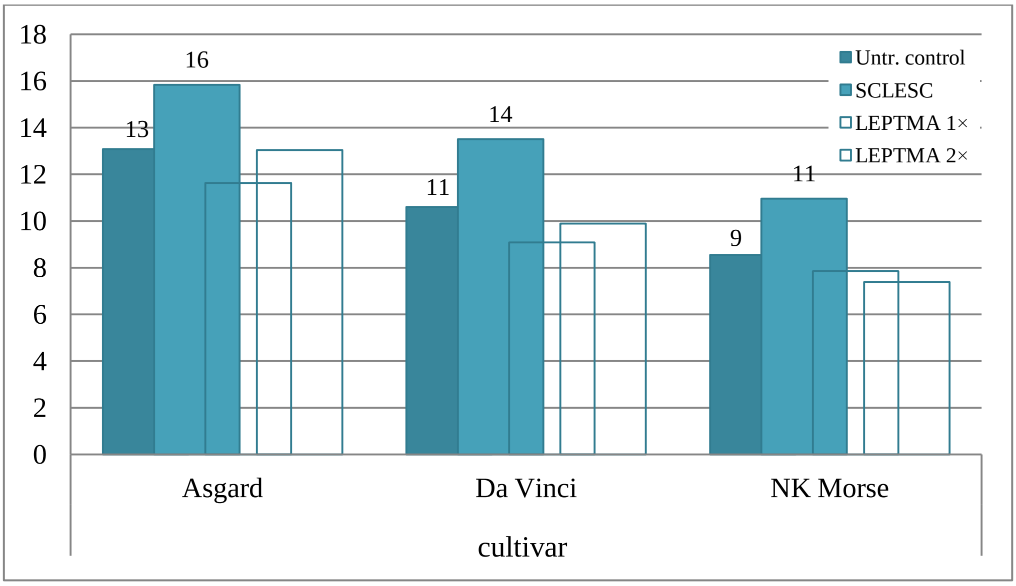

Figure 1. Average disease incidence [%] of *Sclerotinia sclerotiorum* on different cultivars of the untreated control, after artificial inoculation with sclerotia of *S. sclerotiorum* (SCLESC) and foliar application of *Phoma lingam* inoculum in autumn (LEPTMA 1x) and in autumn and spring (LEPTMA 2x).

Phoma lingam

The incidence of blackleg as evaluated before harvest in Opava in 2012 was high, with incidence ranging between 46 to 60%. Increased levels were observed after artificial inoculation only on the susceptible variety Asgard on plots treated once (in autumn) and also twice (in autumn and in spring) with *Phoma lingam* inoculum. In contrast, variety NK Morse only showed increased incidence levels of blackleg on plots treated twice with *Phoma lingam* inoculum. In 2013 the incidence level of blackleg was medium and incidence levels ranged between 31 to 32%. After artificial inoculation of plots the incidence level of blackleg increased at all treatments by 0 to 5%. Results obtained from testing of the susceptible variety Asgard were interesting because the level of blackleg counted from records from plots treated once was higher than in case of records obtained from plots treated twice.

The incidence level of blackleg evaluated before harvest in Šumperk in 2012 was low, incidence level ranged between 10 to 16%. After artificial inoculation with *Phoma lingam* inoculum, no variety response was observed. In 2013 the incidence of disease was equal but lower than the previous year, incidence level ranged between 7 to 8%. After artificial inoculation, the observed level of blackleg increased by between 2 to 12%. The highest level was recorded on the susceptible variety Asgard. Varieties Da Vinci and NK Morse responded equally, where the incidence level of blackleg was 6 and 7% for both varieties. Results are given in Table 2.

On average, from the data obtained for these years and localities, the highest incidence level after artificial inoculation with *Phoma lingam* conidiospores was observed on the susceptible variety Asgard when treated twice (higher by 5%). The variety Da Vinci had no response and the disease incidence of variety NK Morse achieved an increase of 2% after being treated twice (Figure 2).

Table 2. Disease incidence (%) of blackleg on different cultivars of oilseed rape in monitored years and localities of the untreated control (T1-C), after artificial inoculation with sclerotia of *S. sclerotiorum* (T2-S) and foliar application of *Phoma lingam* inoculum in autumn (T3-P1) and in autumn and spring (T4).

Year	Location	Opava				Šumperk			
		Treatment				Treatment			
	Variety	T1-C	T2-S	T3-P1	T4-P2	T1-C	T2-S	T3-P1	T4-P2
2012	Asgard	60	60	65	68	16	10	10	11
	Da Vinci	46	50	37	37	10	9	10	7
	NK Morse	55	51	48	58	10	11	10	8
2013	Asgard	33	35	34	37	8	9	10	20
	Da Vinci	32	33	37	32	7	8	13	14
	NK Morse	31	30	33	33	8	8	15	14

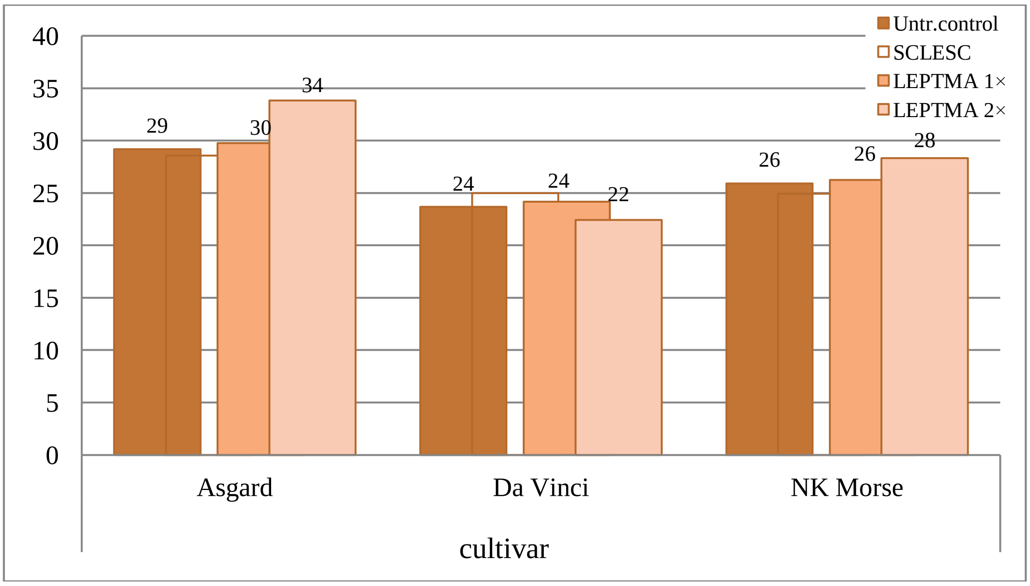

Figure 2. Average disease incidence [%] of the pathogen *Phoma lingam* on different cultivars of the untreated control, after artificial inoculation with sclerotia of *S. sclerotiorum* (SCLESC) and foliar application of *Phoma lingam* inoculum in autumn (LEPTMA 1x) and in autumn and spring (LEPTMA 2x).

The results obtained showed that in the case of artificial inoculation with *Phoma lingam* conidiospores, the incidence level was influenced again by individual years at selected localities and the resistance level of oilseed rape variety. In case of high incidence level, the response of susceptible variety was higher (more severe damage) than the response of the resistant variety. In case of low incidence of blackleg the resistant varieties were affected more than in years with higher incidence level of blackleg.

It was confirmed that infestation of oilseed rape in the Czech Republic strongly depends on local weather conditions in a specific season and on locality (Plachká & Macháčková, 2012), where each site has another natural source of infection, different weather events and different developmental stages of crop.

Conclusion

All varieties of oilseed rape responded to artificial inoculation by *Phoma lingam* and *Sclerotinia sclerotiorum* inocula. We have also noted differences in the response of varieties to artificial inoculation with respect to year and location. Field inoculation with the pathogens *Sclerotinia sclerotiorum* and *Phoma lingam* increased infestation level of oilseed rape overall.

The required infestation level of fungal diseases for acceptable results, with respect to efficacy testing of fungicides, has been set to 5% recorded on untreated control. Based on the results obtained in this study, it can be concluded that in the case of low infection pressure and favorable conditions for disease development, the artificial inoculation with pathogens *Phoma lingam* and *Sclerotinia sclerotiorum* can positively influence the explanatory power of field tests and therefore, the acceptance of results.

Acknowledgement

The results were obtained with financial support of the Technology Agency of the Czech Republic, Programme Alfa, project No. TA 01010815.

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Sclerotinia stem rot

Integrated Control in Oilseed Crops
IOBC-WPRS Bulletin Vol. 104, 2014
pp. 117-125

Interactions between winter oilseed rape canopy structure at flowering and *Sclerotinia sclerotiorum* epidemiology

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Abstract: *Sclerotinia* stem rot is one of the major diseases of winter oilseed rape. It is mainly controlled by fungicide application. Risk occurrence is estimated on petals, through the use of a petal kit. Nevertheless this epidemic potential is often limited by different environmental factors and plant infection capacities are strongly restricted. At the field level canopy structure can modulate conditions more or less able to favour the epidemic. We have looked for canopy structures able to modify disease impact. The initial hypothesis was that a closed canopy could favour the disease development by increasing local relative humidity and temperature. Field trials were carried out during two agronomic seasons with different genotypes and different plant densities. Infection potential, plant branching, petal fall kinetics on different parts of the canopy, wetness and temperature conditions inside the canopy and symptom occurrence was recorded. Results indicated (i) the plasticity of the plant and its ability to compensate at low plant densities, (ii) number of contaminated fallen stuck petals on leaves are not limiting for the epidemic, (iii) microclimatic differences inside and outside the canopy are not so important, (iv) disease incidence was higher for the lowest plant densities for the two locations where we got symptoms. This allows us to arrive at a new hypothesis that has not yet been tested.

Key words: Rapeseed**,** *Sclerotinia* stem rot, canopy architecture, integrated disease management

Introduction

Sclerotinia sclerotiorum (Lib.) de Bary, is one of the major diseases for winter oilseed rape with *Leptosphaeria maculans*. Damaging levels of incidence and severity occur two to three times per decade. The main strategy of protection is fungicide applications at the beginning of petal fall. Nevertheless, these applications are early in the epidemiological process and fungicide applications looks like an insurance measure without proper risk estimation. Occurrence of the disease is much more dependent of the climatic conditions present during leaf penetration and growth of the mycelium inside the plant, from the leaf to the stem. There is strong societal and political pressure to reduce pesticide applications. Nevertheless risks are often overestimated with the different decision making tools available for farmers. Paradoxically to what we expect from a decision making tool, this early risk estimation induces a fungicide application frequency increase. Several alternative strategies are under study. For several years a sclerotia parasitic fungus, *Coniothyrium minitans* was commercialized (Penaud *et al*., 2011). There are several big international consortia working to look for plant resistance to the fungus. We can expect from these programs less susceptible cultivars in a near future. From an agronomic point of view, longer rotations with less susceptible crops should become a possibility. Considering others plant pathogen interactions, canopy structure could have an effect on disease development. For example, there have been studies on peas for *Ascochyta* (Schoeny *et al*., 2010), on soybean for *Sclerotinia* (Grau *et al*., 1984), or for sunflower diseases (Seassau *et al*., 2010; Desanlis *et al*., 2013). In early 90's, effects of plant growth regulators have also been shown to increase occurrence of sclerotinia on winter oilseed rape (Souliac, 1991). A farmer fields diagnosis network carried out by CETIOM in 2000 also supported this conclusion (Caceres, 2000). These results suggest that manipulation of canopy structure could be a way to manage and reduce disease incidence risk. Our objective is to look at interactions between oilseed rape canopy at flowering and *Sclerotinia sclerotiorum* development and identify factors that may limit disease development. The hypothesis was that an open canopy with good air circulation would limit disease development through a reduction in the amount of contact between contaminated petals and leaves, and therefore a canopy microclimate unfavorable for disease development.

Material and methods

Experimental design:

Six field trials in 2009-10 and five trials in 2010-11 were carried out at CETIOM or in partnership with local "chambres d'agriculture" (local official advisory services) (Table 1). Treatments that were compared were combinations of plant density (from 10 to 60 plants /m^2), genotype (hybrids or open pollinated lines), and 15 days delayed second N fertilizer application (without any effect due to dry spring weather). Experimental designs were randomized blocks or split-plot designs with 3 or 4 replications. No fungicide or plant growth regulator was applied.

Table 1. Field trial locations and main treatments

Year	Location	Partner	Plant densities (plants/m^2)	Number genotypes
2009-10	78	CETIOM	10; 30; 60	2
	36	CETIOM	10; 30; 60	2
	21	CETIOM	10; 30; 60	2
	27	Chbre Agric.	30; 60	3
	17	Chbre Agric.	10; 30; 60	1
	79	Chbre Agric.	10; 30; 60	1
2010-11	78	CETIOM	10; 30; 60	2
	21	CETIOM	10; 30; 60	2
	27	Chbre Agric.	15 to 20; 50	1
	17	Chbre Agric.	15 to 20; 50	1
	58	Chbre Agric.	15 to 20; 50	1

Canopy characterization

Plant density and biomass was measured before and after winter following classical procedures (Guide de l'expérimentateur CETIOM) and the experiments were assessed at growth stages BBCH 32, 60 and 70. Leaf area index was measured on two locations, site 78, Grignon (near Paris) and at site 21, Beire le Châtel (Near Dijon) from beginning to the mid-term flowering period (depending on the cultivar earliness). On two replications one square meter was sampled per primary plot. A subsample of 5 plants was used for detailed measurements. Fresh weight is taken for both samples and subsamples. On each plant each stem was measured from the bottom to the top and grouped in three plant height categories (0-30 cm; 30-60 cm; > 60 cm). LAI were measured on a planimeter for each subsample and calculated for one soil square meter.

For all locations, number of secondary stems was registered per 30 cm height segment. This is done at stage BBCH70 on 5 plants per elementary plot from destructive samples in CETIOM and non-destructive ones elsewhere.

Disease epidemy

Petal kits were used at all the trials at flowering, following the procedure described by Poisson-Bamme & Penaud (2000) developed from an initial method described by Morall & Thompson (1991).The measurement provides an estimation of the rate of contaminated flowers which is considered as an estimator of the disease risk. If > 30% of flowers are contaminated, the risk is considered high. Petal kits results were confirmed after a 15 days delay waiting for sclerotia initiation. A second kit test was done at each trial 10 days after when the first result was lower than 50% of flowers contaminated.

Climate data

Climatic data (temperature and relative humidity) were collected from March to June. At trials carried out by CETIOM, small recorders (La Française d'Instrumentation Ref. FI84ED) were used outside and inside the canopy to contrast treatments. These sensors of the recorders were placed at 40cm from the ground and protected from rainfall. Data were recorded every hour.

Epidemiological models

We used the "RAISO-Scléro" model developed by Syngenta (Varraillon *et al.*, 2011). From agronomic and climatic data it provides simulations of the kinetics of petal contamination. The model has been used for the different trials using climatic data from neighbouring MeteoFrance sites.

Symptoms

Observation and quantification of fallen petals was done at different levels inside the canopy. Counts of petals that had landed or stuck were done per 30 cm height segment of plants for each trial and all the treatments. For CETIOM field trials, that was done at growth stage BBCH 70 on 5 plants that were destructively sampled per treatment. For the others trials petals that had fallen and stuck were estimated without plant destruction. A second check has been done with the same method on all the field trials 2 weeks later, with the exception of the Grignon site. In this location a destructive plant sampling was carried out on two adjacent rows of 1 meter length, only on the hybrid NK Aviator.

At the end of the season, at growth stage BBCH 73, diseases symptoms were seen only at two trials, in 2010 in Normandy (site 27 at Eure) and in Burgundy (site 21) in 2011. Symptoms were checked on 8 x 25 plants per primary plot.

Results

Petal kits

For seven of the ten sites *Sclerotinia* spores contaminated petals frequency was high (over 30%) with some variations among sites (Table 2). The latest data site 11 in Normandy in 2011 was not used since there were too much volunteers from a previous oilseed rape crop. When 2 different kits were used at the same site, the results were complimentary with the exception of 2 sites where it seems to have picked up different spore emissions.

Table 2. Results for the petal-kit expressed as the frequency of petals where the Petri dish media colour changed.

Sites 2009-10	**17**	**21**	**27**	**36**	**58**	**78**	**79**
1st kit (date)	45% *(16/04)*	7.5% *(30/04)*	70% *(7/05)*	12.5% *(12/04)*		25% *(19/04)*	37.5% *(26/04)*
2nd kit (date)		17.5% *(4/05)*		5% *(19/04)*		7.5% *(30/04)*	
2010-11							
1st kit (date)	40% *(1/04)*	18% *(11/04)*	*Non validated trial*		100% *(11/04)*	60% *(11/04)*	
2nd kit (date)	45% *(11/04)*	75% *(22/04)*				20% *(22/04)*	

Model

Syngenta's model was used and gave estimations that were very similar to the petal kit results for each site. Data are not shown.

Branching

The number of shoots was dependent on plant density. When plant density was low, then branching was high. We got from 9 to 15 branches at low plant densities, 6 to 11 for intermediate plant density, and from 4 to 8 for the highest plant density. These quantifications underline the well-known plasticity of the crop and its compensation capacity when the environment resources allow it. Compensation was different between sites and environments. This plasticity is illustrated in Figure 1 for the Burgundy site (21) in 2010-11.

Differences were also observed among years. During the second experimental year, secondary branching started from the higher part of the main stem, which was much higher than the previous year, where branching started lower, from the intermediate stem category.

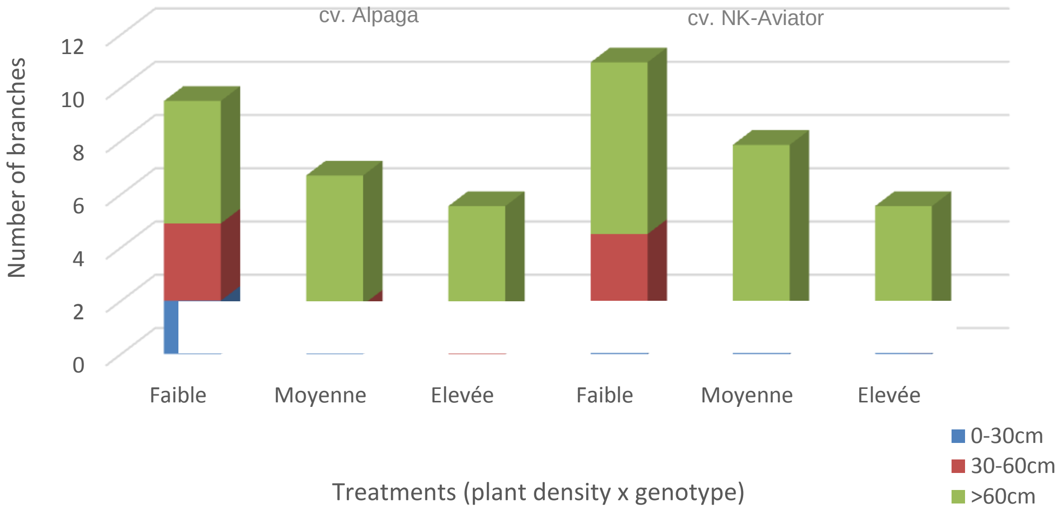

Figure 1. Number of branches along the plant height (blue: 0-30 cm, red: 30-60 cm, green: > 60 cm) for the different plant densities (low: 10; medium: 30; high: 60 plants/m^2) and for the two genotypes (Alpaga, NK-Aviator) at the trial sites in Beire le Châtel (21 Côte d'Or) in 2010-11.

Leaf Area Index (LAI)

Leaf area index was measured per plant height category at flowering for 3 locations: both years at Grignon near Paris (site 78) and in Burgundy during the second year (Beire le Châtel; site 21) (Table 3).

Table 3. Leaf area index (LAI; m^2 leaf/m^2 soil) for three sites of each genotype and for the three plant densities.

	Grignon 2009-10		Grignon 2010-11		Beire le Châtel 2010-11	
	Catalina	NK Aviator	Alpaga	NK Aviator	Alpaga	NK Aviator
Low plant density	2.2	3.5	1.4	2.0	3.0	4.0
Intermediate plant density	3.5	3.6	1.6	2.3	3.2	5.6
High plant density	2.9	3.8	1.8	3.1	3.9	3.2

In Beire le Châtel, we got high values, with the highest for the intermediate plant density. In Grignon the lowest LAI were obtained during the second year after a dry winter. There was a parallel increase of LAI with the plant density, except for NK Aviator in Beire le Châtel and for Catalina the first year at Grignon. In those two cases a higher value was measured for the intermediate plant density. For each site, LAI were higher for the hybrid cultivar, and lower for the classical open pollinated line. In 2009-10 the higher LAI value was generally found for the first plant height segment (0-30 cm) where as for the following year the higher LAI values were found for the intermediate plant height segment (30-60 cm).

Petal sticks

Petals that landed or stuck on plants were difficult to estimate properly. There was a lot of variation caused by differences in measurement periods, plant growth development stages, and from climatic conditions especially concerning wind and rain. During the first experimental year, in 2009-10, at the beginning of flowering, counts were already high, from 62 to 835 petals/canopy m^2. We also got locations effects as counts were significantly lower for low plant densities in two of the four locations. Number of petals was always higher on the lower strata of the canopy. Later, at the end of the flowering period counts were much higher.

During the second year of study (2010-11), we choose to count petals stuck on leaves only from mid to the end of the flowering period. At mid flowering the number of petals stuck ranged from 0.7 to 16.7 petals per plant with highly significant location effects amongst the four sites. At the end of flowering, the number of petals stuck ranged from 5.2 to 85.6 per plant, again with a significant location effect. For three among the four sites, we got more petals stuck per plant for the lower plant densities. For three of the four locations, the petals numbers were significantly higher on the intermediate strata of the canopy (30-60 cm).

Symptoms

Sclerotinia symptoms were only observed at two locations: in Normandy (site 27) in 2009-10 and at Beire le Châtel (site 21, Burgundy) the second year 2010-11. For these two locations we got the highest plant biomass, with the highest LAI measurements. For the other sites, the weather data indicated maximum temperature and relative humidity necessary for infection/symptoms were favorable for a too short time during the study.

Table 4 presents results from the Normandy site (27) during the 2009-10 season for the three cultivars present in the trial. We got significant plant density and genotype effects. The plant densities were clearly distinct and disease incidence was higher for the lower plant densities for the three genotypes. There was also a significant genotype effect, with lower incidence on Alpaga.

Table 4. Disease incidence of *Sclerotinia* for the different plant densities and cultivars in a field trial located in Eure (27), Normandy in 2009-10.

Plant density	Catalina	Albatros	Alpaga
Low	37%	33%	25%
Intermediate	26%	30%	19%
high	23%	17%	17%

For the 20 10-11 cropping season, plant incidence observed in Beire le Châtel (site 21) was much lower (Figure 2). With such low levels of incidence, we increased as the number of plants observed in order to get an accurate assessment of the percentage of plants with symptoms. Similarly to the previous year’s experiment at the Normandy site, the results indicated higher incidence of *Sclerotinia* for lower plant densities.

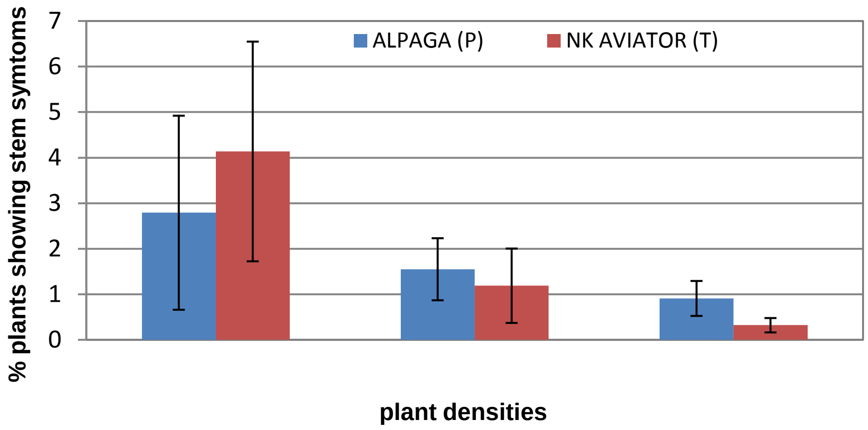

Figure 2. Disease incidence of *Sclerotinia* on stems for the different plant densities (low: 10; medium: 30; high: 60 plants/m^2) and the cultivars Alpaga and NK-Aviator in a field trial located at Beire le Châtel (site 21), Côte d'Or in 2010-11.

Discussion

Winter oilseed rape is a plant which shows undeterminant growth. It is extremely plastic, and able to compensate for low plant densities, increasing branching if the environment allows. A clear demonstration of this plasticity with increased numbers of branches where there is plant density decrease was seen during the study. This ability to compensate reduced the contrast between treatments to a degree that was not expected. Nevertheless, depending on the climatic conditions each year, branches were grown more or less early and from different positions on the main stem. The plasticity we observed on branching can also be expressed through longer flowering periods or during grain filling with higher grain thousand weights.

Sclerotinia spore emissions during flowering period are usual in Western Europe. With a background of twelve years petal kit testing data from all over France, CETIOM has shown that often, with the exception of 2003, petal kit tests results overestimate the thresholds of 30 or 50% which are used to advise regarding fungicide application (Penaud *et al*., 2009). In our results, for 7/10 situations we got a non-limiting presence of pathogen spores.

Nevertheless we got final symptoms only for 2 sites/years (site 27 Eure-Normandy in 2009-10, and Burgundy (site 21 Beire le Châtel) in 2010-11). This suggests that there are a number of steps of major importance in between which could be limitations in the epidemic process, especially climatic factors.

The data on leaf area and on petals that had fallen and stuck suggest that the landing surfaces are not limiting to the infection process. Numbers of fallen and stuck petals on leaves were numerous on each plant and are not a limiting factor.

Looking at climatic data, temperature and relative humidity measured inside or outside the canopy and at different levels of the canopy, we can notice that the epidemiological conditions defined by Brun (1990, 1992) are often not satisfied. This may explain why we didn't see any symptoms at several sites including Grignon where the high relative humidity periods required were satisfied only for short periods after a rain fall event, and often with a quick low air temperature limitation. We observed important variations during day and night for temperature as well as for air relative humidity, outside the canopy. There was no major difference between insides and outside and among the trials contrasted by different treatments. There are only buffered variations inside the canopy.

Contrary to our initial hypothesis, a dense canopy didn't increase incidence or severity of the disease at the final assessment of stem symptoms. An explanation for this could be that there was strong plasticity of the crop or the absence of contrasted temperature or relative humidity levels among the tested treatments. Nevertheless it was surprising to observe that the final results were the opposite of what was expected with more symptoms for the lowest plant densities for two different sites that had high levels of LAI. Our results suggest the following hypothesis: Leaves senescence is dependent on light interception. A leaf receiving intensive sun light will remain active with regard to photosynthetic activity and will therefore see delayed senescence. In contrast, a leaf with restricted sunlight will senesce quicker and will fall earlier. When plant densities are lower, LAI values are limited which allows sun light to filter through the "open" canopy and to reach the lower leaves. The consequence would be photosynthetically active leaves that are retained longer giving enough time for the fungus mycelia to grow through the leaf and reach the stem before leaf fall. With high plant density and higher values of LAI, sun light would be intercepted by the upper leaves. Without enough light, lower leaves would senesce and fall earlier, before the mycelia would have the time to reach the stem.

To test this hypothesis, there was a need to have measured PAR (Photosynthetically Active Radiation) at different heights in the canopy and to have recorded the dynamics of leaf fall. This was not done, and will need to be done in future experiments.

Acknowledgements

This work was done in the framework of the CASDAR project PICOBLE supported by the French Ministry of Agriculture, itself part of the UMT PIVERT program at Grignon campus.

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'SYield' – a risk alert system for *Sclerotinia* in oilseed rape

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Abstract: The fungus *Sclerotinia sclerotiorum*, causes *sclerotinia* stem rot of oilseed rape (OSR, canola) and also infects several other important crops such as sunflower, soya, beans, carrots and lettuce. Spores of this fungus do not infect healthy plant tissue directly as they need to colonise senescing leaves or petals, which provide an energy source, to allow production of the main pathogenicity factor, oxalic acid, which kills healthy tissue into which the pathogen grows. Infection typically occurs when there is a coincidence of airborne spores, a susceptible crop growth stage (flower petal fall) and suitable infection conditions. Although weather-based disease forecasts exist, these are often based only on infection conditions or operate at regional scales. In many countries, including the UK, epidemics are sporadic and have not been well-predicted by current models, which combined with high crop value, leads to many farmers spraying two or three times at flowering. A more precise inoculum-based warning has potential to optimise fungicide spray applications, improving disease control and avoiding unnecessary sprays. The 'SYield' system is an automated 'lab-in-a box' connected to an innovative air sampler that traps airborne particles and incubates them in a suitable semi-selective growth medium. After incubation, an assay for oxalic acid is made using a biosensor. Each day, results from the incubated samples are transmitted wirelessly to a server, along with hourly met data collected from an integrated met station. These results are processed to make a risk prediction, which is texted to the farmer. The system is intended to work as a network of sensors, which will reinforce risk alerts on a regional scale. Satellite image data could also be used to optimise deployment of the sensors, interpreting results based on wind direction and proximity to locations of previous susceptible crops. Initial results in 2012 compared well with results from established Hirst-type spore traps that were operated alongside the prototype units and analysed retrospectively using lab-based qPCR. Results from more extensive field testing in 2013 using several farm and rooftop sampling-sites will be presented. This and similar automated detection of spores of other pathogens offers the potential for precision disease control as part of integrated pest management.

Key words: inoculum warning, IPM, miniature virtual impactor, networked sensor, biosensor

Light leaf spot

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IOBC-WPRS Bulletin Vol. 104, 2014
pp. 129-133

Light leaf spot (*Pyrenopeziza brassicae*) – a resurgent problem in the UK

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Abstract: Since 2006, the incidence and severity of light leaf spot (*Pyrenopeziza brassicae*) in winter oilseed rape in the UK has increased so that the disease is now the main disease concern for growers with respect to yield loss. For example, in the springs of 2011, 2012 and 2013, > 70% of crops and > 30% of plants surveyed in England were affected by the disease. Crops have been severely affected in southern England as well as further north. It is thought that the increase in the prevalence of light leaf spot may be due to factors such as recent unusual weather conditions, a lack of resistance in currently grown varieties and poor disease control from poorly timed fungicide applications. Seasonal guidance for growers has been provided for more than 10 years by the on-line light leaf spot forecast but there is a need to refine predictions of epidemic onset in autumn to improve fungicide timing and overall disease management. National survey data highlight the need for new research to investigate the reason for this change in disease prevalence. This paper describes our current understanding of light leaf spot and work to be done in two new projects. One project aims to investigate pathogen development/epidemic onset in autumn and the role of subsequent components of the epidemic in epidemic progression. The other (PhD) project aims to investigate aspects of the light leaf spot pathogen populations with respect to improving varietal resistance.

Key words: Apothecia, ascospores, *Brassica napus*, conidia, *Cylindrosporium concentricum*, epidemiology, forecasting, modelling, spore trapping, vegetable brassicas

Introduction

Light leaf spot, caused by *Pyrenopeziza brassicae,* is a disease that causes considerable yield loss in winter oilseed rape (*Brassica napus*) in the UK (Fitt *et al*., 1997; Karolewski *et al*., 2002). It is a polycyclic disease that is favoured by cold, wet conditions. This is in contrast to the other major oilseed rape disease, phoma stem canker (*Leptosphaeria maculans*) that is monocyclic. Light leaf spot has been a problem for oilseed rape growers in northern England and Scotland in the past (Welham *et al*., 2004). The pathogen infects oilseed rape leaves, stems, flowering structures and subsequent pods from the time the crop is autumn-sown through to harvest the following summer (Gilles *et al*., 2000). The main effect of the disease on yield is through decreased leaf photosynthetic area and plant vigour and increased frost susceptibility in winter (Baierl *et al*., 2002).

Effective control of light leaf spot can be achieved only when fungicides targeted at the initial infections are applied in autumn (fall) (Figueroa *et al*., 1994) and protection is maintained thereafter. However, as infections are often symptomless at this time, crop-specific risk forecasts can be invaluable to guide fungicide spray decisions (Evans *et al*., 2004; Welham *et al*., 2004). The Rothamsted light leaf spot forecast produces a regional, crop-specific forecast of the incidence and severity of light leaf spot that a grower in a particular region can expect to observe in crops the following spring, at the time in the autumn when crops may need to be sprayed. This provides the grower with an indication of whether an epidemic is likely to occur during that growing season in a given region (http://www.rothamsted.ac.uk/light-leaf-spot-forecast). In contrast, the phoma leaf spot forecast, hosted on the same Rothamsted website (http://www.rothamsted.ac.uk/phoma-leaf-spot-forecast) provides growers with a weather-driven forecast of the day in autumn when 10% of plants at a particular location can be expected to show leaf spotting. Ten percent of plants with leaf spotting is the industry agreed threshold that triggers a decision on application of a fungicide targeted at phoma leaf spot (Evans *et al*., 2008). It would be beneficial if a similar forecast could be developed for the date of onset of light leaf spot epidemics, since this would provide more specific information to growers about fungicide application timing.

Recent trends in the most damaging UK oilseed rape diseases

For many years, phoma stem canker (*Leptosphaeria spp.*) has been the predominant disease in the main oilseed rape growing regions of the UK. In England, for example, it caused losses of up to £ 140 million in 2006 (Figure 1). In contrast, light leaf spot was considered to be problematic only in northern England and Scotland, where as English annual losses were estimated to be £ 10-40 million per annum (Figure 1). However, annual survey data from the Defra winter oilseed rape survey show that over the past six seasons the trend has reversed with phoma becoming less of a problem in England (Figure 1) and light leaf spot increasing in incidence and severity (Figure 2; www.cropmonitor.co.uk). With regard to the increase in prevalence of light leaf spot, Figure 3 indicates that the incidence and severity of light leaf spot was high in all regions in spring 2013.

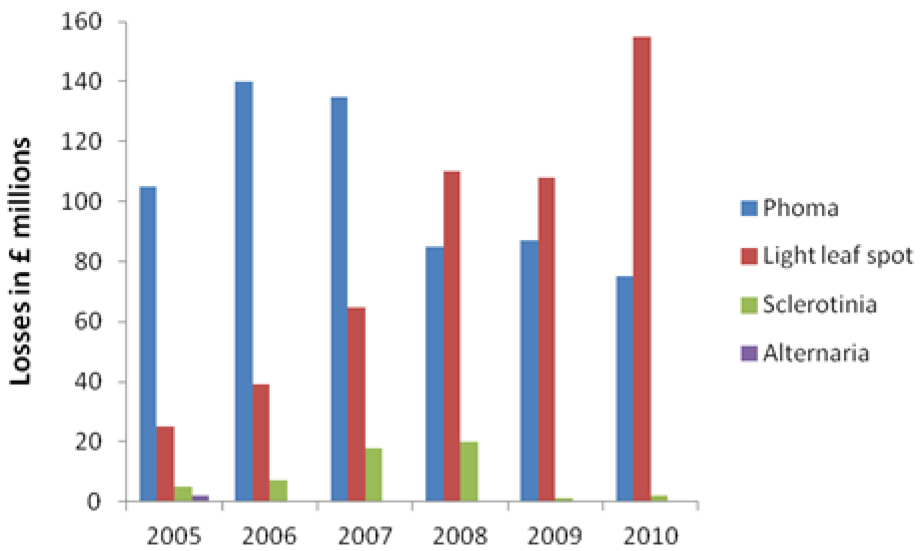

Figure 1. Losses (£ millions) attributed to the four main winter oilseed rape diseases in England, indicating changes in prevalence of the two main diseases, phoma stem canker and light leaf spot. Data from www.cropmonitor.co.uk

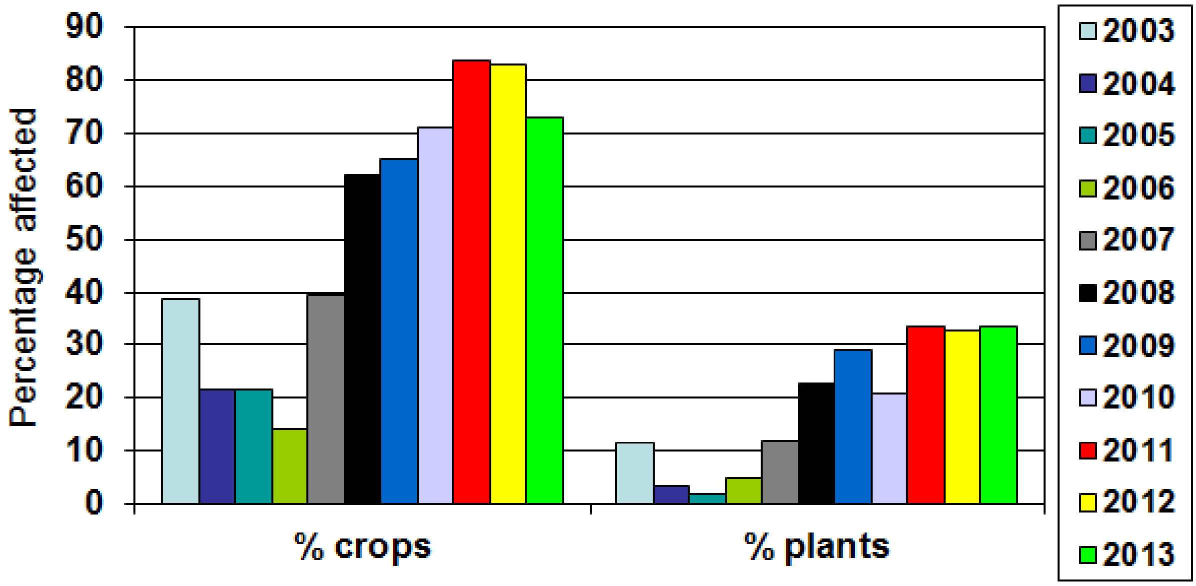

Figure 2. Recent annual increases in the incidence of light leaf spot in commercial crops (percentage of crops and percentage of plants affected) at early stem extension (March) in England 2004-2013. Data from Defra-funded winter oilseed rape survey (source: www.cropmonitor.co.uk)

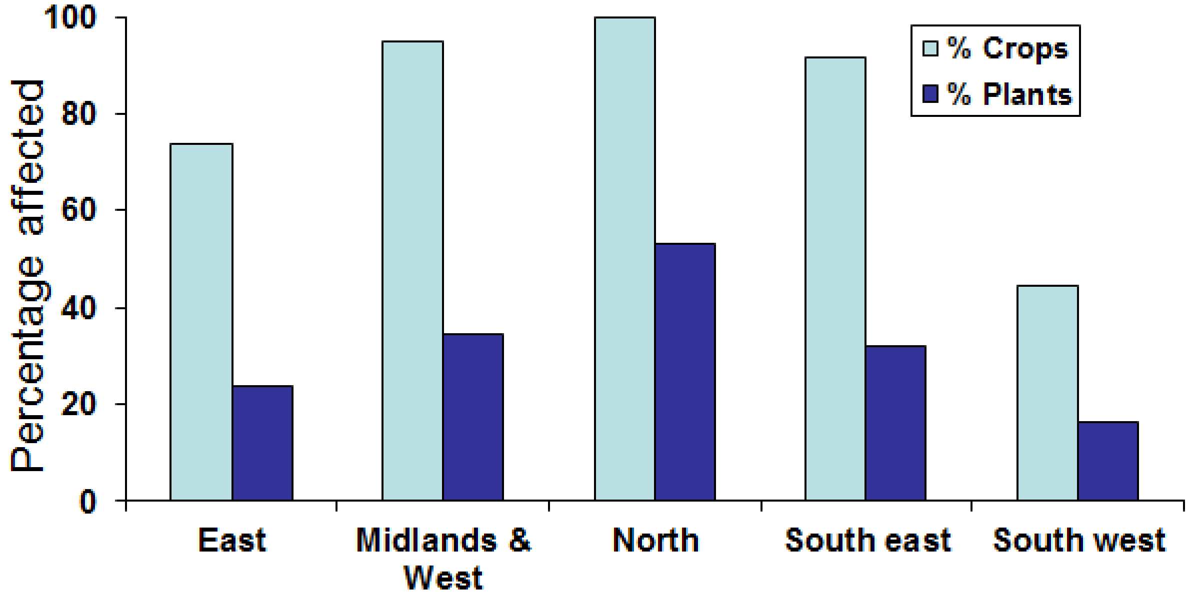

Figure 3. Regional variation in the percentage of crops and the percentage of plants with light leaf spot in England during April 2013. Data from Defra-funded winter oilseed rape survey (source: www.cropmonitor.co.uk)

There is no simple explanation for the sustained increase in light leaf spot severity. Phoma stem canker can be controlled very effectively by fungicide sprays in the autumn and early winter but these treatments are not providing good control of light leaf spot. Within the agricultural industry, there have been many hypotheses, such as recent occurrence of cold winters with snow that may have hindered *L. maculans* growth whilst they have favoured light leaf spot. It is difficult to identify the early stages of epidemics of light leaf spot and many farmers have been applying fungicides to severely affected crops. This is too late to achieve good control (Gladders *et al*., 2009).There have been reduced spray programmes for phoma in years with late epidemics or difficult ground conditions for spray application and these may have allowed light leaf spot epidemics to develop more rapidly. However, there is a need to study the early develoment of light leaf spot to understand the recent increase in its prevalence and to provide growers with solutions to control this disease.

New research on light leaf spot

Investigating components of the oilseed rape light leaf spot epidemic responsible for increased yield loss to the UK arable industry (HGCA 3814)

The main objective of the project is to develop a novel decision support tool to predict light leaf spot epidemic onset by modelling the inter-crop development and maturation of *P. brassicae* using pre-defined parameters readily available from the literature. This will provide growers and advisors with a reliable warning that the start of the epidemic is imminent, much like the current phoma leaf spot forecast. The model predictions will be validated by PCR-based analysis of spores collected in existing multi-site air samples from England and Scotland and in new air samples collected from partner project field sites. The models will also be used with disease and meteorological data from a number of the HGCA Recommended List field sites to utilise data from the newly installed HGCA meteorological station network. In addition, remote "in-field" sensing capabilities of one project partner will be used to monitor and record "within crop" environmental and leaf wetness data during the growing season, specifically to investigate whether extreme winter conditions encourage epidemic development, utilising artificial snow, where possible, to experimentally re-create the harsh winter weather conditions encountered in the UK in the past few seasons. The project is led by Weather INnovations (co-ordinator, weather monitoring, modelling), with SRUC (disease monitoring Scotland, spore trapping Scotland, qPCR Scottish spore trap tapes), ADAS (disease monitoring England, spore trapping England), Rothamsted Research (spore trapping England, qPCR English spore tapes) and Bayer CropScience Ltd. providing technical support and industrial sponsorship.

***Improved management of light leaf spot in Brassicas by understanding variation in* Pyrenopeziza brassicae**

This (PhD) project aims to study variation in the pathogen population and will investigate the race structure of the population throughout the UK. The variation between *P. brassicae* isolates will be examined morphologically in culture as well as on a molecular basis using neutral DNA markers. Therefore, isolates will be obtained from both oilseed rape and vegetable brassicas (Brussels sprouts, cabbage etc.) from different sites in the UK to achieve a representative collection of isolates. This collection will also be used for testing oilseed rape and vegetable brassica cultivars to examine plant-pathogen interactions and identify material with resistance and to develop a differential set of cultivars to further characterise *P. brassicae* isolates. Cultivars where the resistance has been rendered ineffective by changes in the pathogen population will be particularly useful for defining major resistance genes and races of the pathogen. Furthermore, the potential for general and selective spread of the pathogen populations between oilseed rape and vegetables brassicas will be investigated by cross-inoculation of brassica seedlings in controlled environment experiments.

Acknowledgements

We thank HGCA and Bayer CropScience Ltd. for funding the project regarding light leaf spot epidemics and HGCA for in-kind support of the PhD project. We are also grateful for funding of the PhD project by Felix Thornley Cobbold Agricultural Trust, HDC and University of Hertfordshire and in-kind support from HDC, ADAS, Elsoms, Limagrain and Trialsforce.

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Blackleg, stem canker

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IOBC-WPRS Bulletin Vol. 104, 2014
pp. 137-142

Molecular detection of pycnidiospores of *Leptosphaeria maculans* from tapes from spore samplers

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Abstract: Oilseed rape in Poland is exposed to two fungal pathogens, *Leptosphaeria maculans* and *L. biglobosa*, which cause stem canker of crucifers and are responsible for considerable yield loss. Considerable reduction of seed yield is attributed primarily to stem infection with *L. maculans*. The primary source of disease are airborne ascospores and secondary infection is caused by fungal pycnidiospores. Modern decision support systems that allow us to forecast the disease are based on the detection of ascospores using volumetric air samplers, but little is known about the detection limits of the secondary inoculum. Analysis of presence and concentration of airborne fungal spores may be performed either by means of conventional microscopy methods or PCR-based molecular techniques. This experiment was designed to optimise methods used for molecular detection of pycnidiospores of *L. maculans* from tapes obtained from spore samplers and processed in the same way as for the detection of primary inoculum. The sensitiveness of two methods was compared: traditional end-point PCR and Loop-mediated Isothermal Amplification of DNA (LAMP). The detection was done using pathogen DNA extracted from vaseline-coated Melinex tapes routinely used in 7-day volumetric spore samplers of the Hirst-type. The sensitiveness of the end-point PCR was 100 pycnidiospores and 50 spores using LAMP. Lower numbers of spores were also occasionally detected. The paper for the first time describes the use of the LAMP method for the detection of the secondary inoculum of *L. maculans* from tapes in volumetric spore samplers.

Key words: *Leptosphaeria maculans,* ascospore, pycnidiospore, spore trapping, molecular detection, Loop-mediated Isothermal Amplification

Introduction

Ascomycete fungi *Leptosphaeria maculans* (Desm.) Ces. et de Not. and *Leptosphaeria biglobosa* Nov. are damaging pathogens of oilseed rape (*Brassica napus* ssp. *oleifera*) and other cruciferous plants worldwide. *Leptosphaeria maculans* causes phoma leaf spotting and stem canker (blackleg) and is responsible for major yield loss, 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). The pathogen survives on plant debris. Pseudothecia – fungal fruiting bodies of the perfect stage – are formed on infected stubble 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 petioles into the stem (Hammond *et al*., 1985; West *et al*., 1999 and 2001). Colonization of stem bases and the root collar is enhanced by high humidity and mild temperatures and can lead to severe plant damage or even plant death. Optimized fungicide spray timing is one of helpful tools in controlling stem canker and protecting crops from *Leptosphaeria* sp. (Gladders *et al*., 1998; West *et al*., 2000; Huang *et al*., 2005). In order to improve stem canker management in Poland, the System for Forecasting Disease Epidemics (SPEC) was developed (Jedryczka *et al*., 2004). The system uses nine to ten volumetric spore samplers to monitor for the presence of ascospores at ten different locations all over the country. The spray time of the fungicide are optimized based on the presence or absence of ascospores of *L. maculans* in air samples (Kaczmarek *et al*., 2014b). A fungicide spray was most efficient, when applied 4 to 14 days after the highest concentration of *Leptosphaeria* ascospores was observed. An application of fungicide at this time reduced the incidence of phoma leaf spotting by up to 25%, stem canker symptoms by up to 33% and decreased yield loss by up to 5 dt/ha.

Conventional method of monitoring for airborne fungal spores involve light microscopy. The method is time consuming and requires experienced personnel. PCR-based molecular techniques in conjunction with traditional spore traps have great potential for detecting airborne fungal spores. Specific primers have been described for *L. maculans* as well as its variants with avirulence alleles (Kaczmarek *et al*., 2014a). This paper reports on experiments established in order to optimise methods used for molecular detection of *L. maculans* from spore tapes obtained from samplers. The aim of the experiment was to compare the efficiency of the detection of pycnidiospores of *L. maculans* using traditional end-point PCR and the new technique of Loop-mediated Isothermal Amplification (LAMP) of DNA.

Material and methods

Fungal isolates and spore suspensions

The experiment was done using isolates of *L. maculans* from Germany, Poland, Sweden and the UK collected during the period 1998-2013. The specificity of the primers was checked using the isolates belonging to the following genera: *Alternaria*, *Aspergillus*, *Botrytis*, *Colletotrichum*, *Epicoccum*, *Fusarium*, *Pyrenophora*, *Rhizoctonia* and *Sclerotinia*. These isolates were obtained from oilseed rape (spring and winter type), pea, lupin, wheat and field bindweed.

Isolates of *L. maculans* were cultured for two weeks and then pycnidiospores were collected and suspended in deionised water. Concentration of pycnidiospores was calculated using a haematocymeter. Pycnidiospores of a measured concentration were randomly deposited on a piece of 19 x 48 mm Melinex tape covered with a thin layer of a mixture of petroleum jelly (vaseline), paraffin wax, phenol and hexane. A piece of each tape with a given concentration of pycnidiospores was used for DNA extraction, in 20 replicates.

Spore disruption and purification of DNA

Fungal DNA was isolated from pure spore suspension in deionised water as well as from Melinex tape. Spores were disrupted by shaking 200 µl of spore suspension together with beads, using a FastPrep machine (Savant Instruments, Holbrook, New York, USA). To efficiently disrupt the spores the process was performed twice for 40 seconds at the speed of 5 m/s with 2 min cooling on ice. DNA was purified from 50 µl of the disrupted spore suspensions using a modified phenol-chloroform method (Lee & Taylor, 1990). The samples were examined under the light microscope before and after processing in a FastPrep machine and the extraction process followed phenol-chloroform protocol.

Primers used in end-point PCR

Specific detection of *L .maculans* DNA was achieved using PCR specific forward primer LmacA and reverse primers LmacRev (Calderon *et al.*, 2002) or a newly designed primer LmR developed especially for *L.maculans*. Specificity of primers was checked with PCR using either previously described LmacA and Lmac Rev primers (Calderon *et al*., 2002) or the newly designed reverse primer LmR.

PCR conditions and DNA visualisation

PCR reactions were done in a 10 µL reaction volume, using 2 µL target DNA. Cycling conditions were 94 °C for 2 min, followed by 39 cycles of 94 °C for 30 s, 65 °C for 30 s and 72 °C for 1 min, with a final extension step at 72 °C for 5 min. In all cases 10 µl of PCR products were analyzed on 2% agarose gels containing 0.5 µg/ml ethidium bromide and the electrophoresis separation was performed for 60 min at 150 V.

Loop-mediated Isothermal Amplification of fungal DNA from pycnidiospores

The tapes containing pycnidiospores of *L. maculans* and DNA was extracted from pycnidiospores from pure cultures were prepared as described above. Each of the following spore concentrations was prepared in 10 replicates: 10, 50, 100, 1000 and 10000 of pycnidiospores per tape. The LAMP assay was carried out in a reaction mixture containing: 1.6 µM each of FIP and BIP primers, 0.2 µM each of F3 and B3 primers and 0.8 µM each of LF and LB primers, 1 µl of template DNA (10 ng/µl), Isothermal Mastermix - fluorescent Dye 1x (Optigene, UK) and sterile deionized water, filled up to the final volume of 10 µl per sample. The primers were designed using LAMP primer designing software http://primerexplorer.jp. To perform the reaction, the above listed mixture was incubated in a thermoblock at 64°C for 60 minutes. Termination of the reaction was done by heating at 85 °C for 2 minutes. Visual detection of products was done using Genie II Ultra Rapid Gene Amplificator (Novazym Polska).

Results and discussion

Specificity of primers

Specifity of primers was checked using spores of both *L. maculans* and *L. biglobosa* against a background of spores of 13 other species, including spores of *Alternaria, Epicoccum, Botrytis, Rhizoctonia, Colletotrichum, Fusarium, Sclerotinia* as well as *Aspergillus sp*. All pairs of primers were species specific. Only in the presence of *L. maculans* DNA in the sample and LmacA and LmacRev or LmR primers, one band of a specific product was visible on the gel. Type of reverse primer, either *L. maculans* specific LmR or specific for both *Leptosphaeria* spp. LmacRev, did not influence the specificity of reaction and in both cases obtained product was of the same intensity. There were three negative controls, namely a piece of a spore tape without any DNA deposited on it, premix without any DNA added and a mixture of *Alternaria* and *Sclerotinia* DNA and none of them resulted in a PCR product.

Resolution of DNA detection of a PCR product in a pycnidiospore suspension

A full ITS1-5.8S-ITS2 fragment was obtained using universal primers PN10 and WIRZ G1 (Irzykowski *et al*., 2004). The size of this product for *L. maculans* was 566 bp. Experiments with dilutions were carried out to estimate the sensitivity of detection as well as the efficiency of the DNA extraction method. For *L. maculans*, the sensitiveness of the method was 8 pycnidiospores in the water suspension (Figure 1).

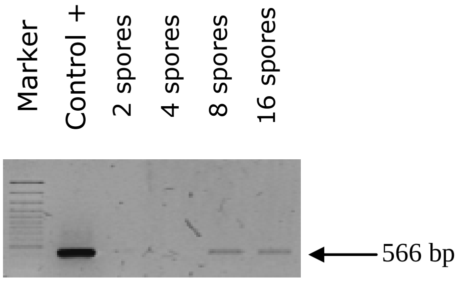

Figure 1. Products obtained by PCR using *Leptosphaeria maculans* pycnidiospore suspensions with known numbers of spores

In the case of the end-point PCR, the lowest detectable amount of *L. maculans* DNA from fresh tape was 100 pycnidiospores. It must be stressed that all spore detections were done from pycnidiospores, which are 200 times smaller than ascospores and contain one cell. Mature ascospores are large and contain six cells, which makes them easier to detect by microscopy and molecular methods. Successful microscopic and molecular detection of airborne ascospores of *L. maculans* has been done in the UK (Calderon *et al*., 2002), Poland (Kaczmarek *et al*., 2009, Kaczmarek *et al.*, 2012) and Lithuania (Piliponyte-Dzikiene *et al*., 2014).

The detection using Loop-mediated Isothermal Amplification was successful when the number of spores per tape was at least 50 pycnidiospores per tape (Figure 2). Occasionally, the detection of 10 spores per tape was achieved, however the detection was successful in 10% of tested samples only.

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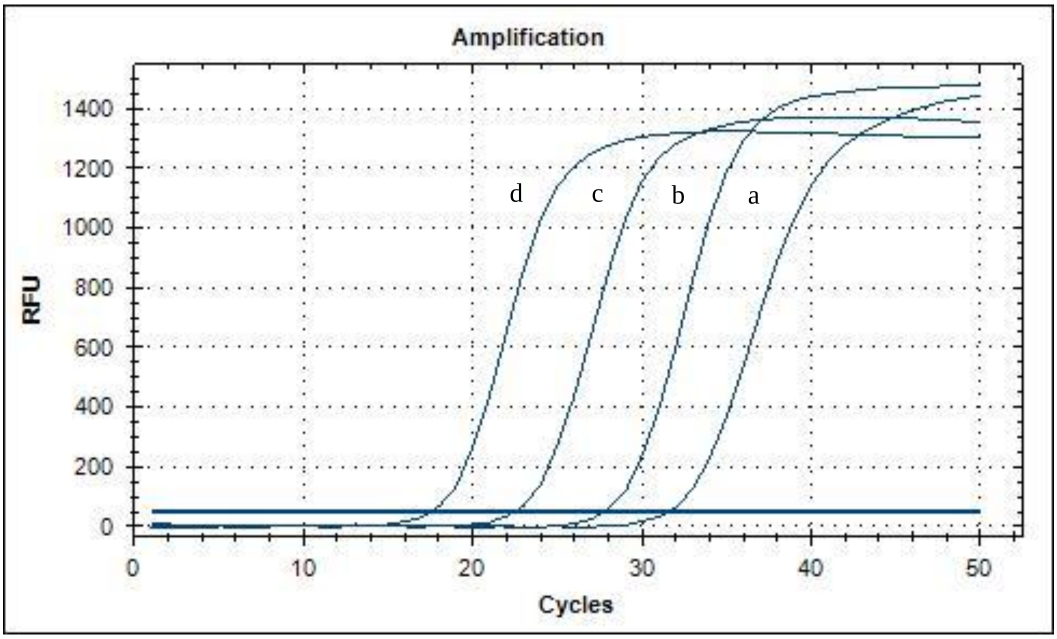

Figure 2. Fluorescence of amplified DNA obtained using different concentrations of pycnidiospores of *Leptosphaeria maculans*: 50 (a), 100 (b), 1000 (c) and 10000 (d).

LAMP is a new technique that offers simple and cost-effective amplification of nucleic acids (Notomi *et al.*, 2000). The method is rapid, but very specific, due to the use of special enzymes and primers. Moreover, the reaction takes a short time and is performed at one temperature in a thermal block, with no need for thermal cycling (Mori & Notomi, 2009). The technique has been shown to be useful for the detection and identification of plant pathogens (Tomlinson & Boonham, 2008; Vincell & Tisserad, 2008). Loop-mediated Isothermal Amplification has been used to study changing *Leptosphaeria* populations in oilseed rape plants and air samples containing ascospores (Jedryczka *et al*., 2013). This paper is the first report on the successful use of the LAMP method to detect pycnidiospores of *L. maculans* from vaseline-coated Melinex tapes.

Knowledge about the concentration of pathogen inoculum in the air allows more efficient protection of oilseed rape against stem canker (Kaczmarek *et al*., 2014b). This paper shows that secondary inoculum of *L. maculans* is also detectable in reasonable amounts using this new, simplified, yet sensitive and cost effective molecular technique. The LAMP test may serve as an additional tool in protection of oilseed rape against stem canker of brassicas.

Acknowledgements

The authors thank to the National Research Centre for funding of the project N N310 298439 for partial funding of this experimental work.

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Detection of *Leptosphaeria maculans* races on winter oilseed rape in different geographic regions of Germany and efficacy of monogenic resistance genes under varying temperatures

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Abstract: Blackleg disease, caused by *Leptosphaeria maculans* is one of the most important fungal diseases in oilseed rape production word-wide (Fitt *et al*., 2006). Genetic resistance is an important tool to control this disease. Seedling resistance is conferred by single major genes. Due to its sexual propagation, *L. maculans* isolates evolve rapidly from avirulent to virulent strains on cultivars harbouring major resistance genes. Therefore, resistance of oilseed rape against *L. maculans* conferred only by major resistance genes was often overcome and led to severe yield losses in the past in France and Australia (Rouxel *et al*., 2003; Sprague *et al*., 2006). Therefore, we cultivated two oilseed rape (OSR) cultivars in 4 different geographical regions in northern Germany in the growing seasons 2011/12 and 2012/13: i) one cultivar harboring no known major gene against *L. maculans* (Lirabon) and ii) one resistant cultivar (Exocet), harboring the major gene *Rlm7*. In autumn and spring we collected true leaves with typical Phoma lesions to gain isolates of *L. maculans*. Isolates obtained from leaves of Lirabon were considered to represent the whole range of virulent isolates in a region. Single spore isolates were tested on a French differential set consisting of 7 OSR genotypes with known major resistance genes for the presence of the avirulence genes *Avr1*, *2, 3, 4, 7* and *9* in a cotyledon inoculation test. Thereby, the frequency of virulent isolates in a region was determined. Isolates gained from Exocet were considered to represent the frequency of *Rlm7* resistance breaking isolates, which was tested in the cotyledon inoculation test with a *Rlm7* harboring cultivar (Caiman). The frequency of virulent isolates on *Rlm1, 2, 3, 4* and *9* was very high with over 80%. The frequency of virulent isolates on *Rlm7* was very low (< 5%). We assume that choice of cultivars with a different complementarity of resistance genes leads to a different spectrum of virulent isolates per region. Furthermore we tested the efficacy of major resistance genes against *L. maculans* under varying temperatures for cotyledons and stems in controlled-environment experiments. Therefore, the resistant cultivars Caiman with *Rlm7* resistance and Uluru with *LepR3* resistance as well as Lirabon as susceptible control were used. For each resistant cultivar an avirulent and a virulent *L .maculans* isolate were selected. Cotyledon resistance was tested with spore suspension, whereas adult resistance was tested at the stem base by inoculation with a mycelium plug. The plant-pathogen interactions were examined at different temperature regimes. Incompatible interactions found on cotyledons of Uluru turned to be compatible, whereas only an increase of *L. maculans* DNA was found for cotyledons of Caiman at higher temperatures (≥ 27 °C). Major gene resistance actively reduced disease severity in stem tissue. Especially Caiman was strongly dependent on its *Rlm7* resistance gene, whereas resistance of Uluru relied more on quantitative resistance. High temperature treatment did not change incompatibility into compatibility at stem bases.

Key words: *Leptosphaeria maculans*, race distribution, efficacy, major resistance genes, Rlm7, LepR3

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Evolution of *Leptosphaeria maculans* populations in a small area of the region Centre (France) following the introduction of oilseed rape hybrids carrying the *Rlm7* specific resistance gene

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Abstract: The specific resistance to blackleg conferred by *Rlm7* has been used in commercial oilseed rape cultivars since 2004. Varieties carrying *Rlm7* have since become wide spread today with a very large market share at the national level. In order to evaluate consequences of the selection pressure exerted by the resistance gene on populations of *Leptosphaeria maculans* (causal agent of blackleg), samples of isolates of *L. maculans* were collected for nine years on plants both with and without *Rlm7* in a small area in the central region of France. Changes in frequency of virulent isolates towards *Rlm7* in these samples as well as changes in the methods used for this analysis are presented.

Key words: stem canker, oilseed rape, virulence, resistance breakdown, molecular diagnostic

Verticillium and other vascular diseases

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IOBC-WPRS Bulletin Vol. 104, 2014
pp. 149-156

Verticillium longisporum: pathogen detection, diagnostics and cultivar resistance in U.K. oilseed rape (*Brassica napus*)

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Abstract: *Verticillium longisporum* is an emerging pathogen of oilseed rape (*Brassica napus*) in the U.K. and both incidence/distribution of the disease appears to be increasing. The delay in the expression of symptoms in the host and the soil-borne nature of the pathogen hamper accurate detection and diagnosis. Chemical control strategies are currently ineffective and rotational management is the only option to minimise risk of disease. The identification of sources of genetic resistance is therefore a desirable strategy through which to reduce disease. In an effort to identify strategies to manage the risk posed by *V. longisporum* we generated a series of inoculated trials to screen a range of current U.K. varieties for resistance and used a CTAB/silicon dioxide pre-extraction, followed by conventional extraction to obtain DNA from a range of soil samples for subsequent analysis. Development of Loop Mediated Isothermal Amplification (LAMP) and qPCR based assays have enabled us to accurately detect and diagnose *V. longisporum* in a range of naturally and artificially infected soil samples. *B. napus* varieties varied for incidence of the disease and although complete resistance would appear to be absent in the varieties screened, a range of partial resistance was observed in specific cultivars. Detection assays currently provide semi-quantitative data for respective DNA concentrations and low (< 50 microsclerotia/50 g soil), moderate (< 100/50 g) and high (> 200/50 g soil) levels of infection can be detected. The identification for variation in resistance to *V. longisporum* amongst current varieties and the development of new diagnostic techniques will be utilised to investigate pathogen dynamics, yield loss and also to develop strategies to manage the risk posed to growers.

Introduction

Verticillium longisporum is an ascomycete fungus affecting a range of *Brassica* crop species. The pathogen produces large quantities of melanised, microsclerotia beneath the epidermis and throughout the inner cortex of the stems of infected *Brassica napus* host plants at the end of the growing season (Karapapa *et al*., 1997). Microsclerotia, with an appearance similar to iron-fillings, are disseminated on the stubble of infected plants. They persist in the soil and the risk of infection posed to subsequent Brassica crops remains prevalent for many seasons.

Although a familiar pathogen in *Brassica* growing regions of Northern European *V. longisporum* was only identified on oilseed rape (OSR) in the U.K. for the first time in 2007 (Gladders, 2009). It is thought *V. longisporum* was introduced on contaminated seed from Continental Europe where seed is multiplied. The continued influx of new inoculum each year is thus likely. Presently knowledge on the incidence/distribution, potential impacts on yield and control of this pathogen is under developed.

No chemical control strategies have yet been identified against *V. longisporum* and information on the levels of disease resistance in the most widely cultivated *B. napus* varieties is absent. The current control method available to growers to combat outbreaks of *V. longisporum* is to abandon OSR cultivation on the infested land for a number of seasons. Therefore, strategies to both reduce the risk of introducing *V. longisporum* (onto the farm) and also how to monitor/control established infestations are of key interest to U.K. growers, especially where intensive wheat/OSR rotations have been adopted.

Quantitative PCR and isothermal DNA amplification techniques are excellent tools to investigate the presence of *V. longisporum* accurately and rapidly in infested soils and seed samples. The ability to quantify pathogen DNA in samples could potentially enable the development of models to predict risk for growers, to limit the spread of disease and to investigate the long term effects of the pathogen on OSR production.

This paper presents results on a varietal screen in a range of U.K. OSR cultivars and the initial findings on the development of a range of assays to identify *V. longisporum* using isothermal and qPCR based techniques.

Material and methods

***Cultivar resistance screen in* B. napus**

Replicated trials (3 row x 2 m plots) inoculated with *V. longisporum* were used to screen for differences in resistance in 18 current OSR varieties. Trial plots were inoculated with an infested maize meal and vermiculite substrate after sowing in 2011/12. Disease incidence was scored as the percentage of affected stems from a total of 30 stems from the centre row of a plot. Scoring was conducted when disease symptoms had developed sufficiently (late July-early August 2012) and visible striping of the stem/production of microsclerotia was observed.

Extraction of DNA from soil

Artificially inoculated 50 g soil samples with microsclerotia at low (< 50), medium (< 100) and high (> 250) loads were prepared for DNA extraction. Triplicate, 50 g soil samples from inoculated plots and 50 g control samples from un-infested land at NIAB, Cambridge, U.K. were also collected. All soil was dried for a minimum of 48 hours at 65 °C before processing. DNA was extracted from soil samples using a modified crude pre-extraction commercial kit and post-extraction clean up (as described by Woodhall *et al.*, 2012, Wizard Food Kit, Promega, U.K.) Samples were ground in a pestle and mortar in 100 ml of buffer until homogenised. A 10 ml aliquot of the slurry was then processed according to the original protocol. DNA was eluted in 100 µl TE and 1 µl samples of the extract used for DNA amplification.

Identification of V. longisporum using isothermal and qPCR

Primers were designed around rRNA sequences to detect *V. longisporum* using Loop-mediated Isothermal Amplification (LAMP), SYBR green and Taqman technologies (Wood, Lee and Thomas, unpublished). Beacon designer software was used to identify suitable Taqman Probes and Primers (Premier Biosoft, U.S.A).

Standard LAMP reactions consisted of 6 µl master mix, 1 µl primer mix, 1 µl DNA extract, and 2 µl ddH20. Reactions were mixed thoroughly, centrifuged briefly and then heated at 65 °C for 45 minutes on a Genie II (Optigene, U.K.).

Standard SYBR Green reactions consisted of 5 µl Maxima master mix (Thermo, U.K.), 1 µl DNA extract, 2 µl primer mix (3 µM), 1 µl ddH20. Standard Curves were generated using *V. longisporum* DNA diluted to 2 ng/µl, 200 pg/µl, 20 pg/µl, 2 pg/µl, 200 fg/µl, 20 fg/µl and 2 fg/µl. 10 µl reactions were loaded onto a 384 well plate, sealed, agitated and centrifuged briefly at 1,000 rpm. Plates were cycled on an ABI7900HT and used SYBR Green/ROX detectors. Reactions were cycled with an initial denaturation at 96°C for 10 mins followed by 40 cycles of 96 °C for 15 secs, 60 °C for 30 secs and 72 °C for 30 secs, followed by a final extension at 72 °C for 10 minutes and dissociative curve analysis.

Standard Taqman reactions consisted of 5 µl Universal Master Mix II (Life Technologies, U.K.), 1 µl DNA extract, 2 µl primer mix (3 µM), 1 µl probe (2.5 µM) 1 µl ddH20. Reactions (10 µl) were loaded into a 384-well plate, sealed, agitated and centrifuged briefly at 1,000 rpm. Plates were cycled on an ABI7900HT and used FAM-6/ROX detectors. Reactions were cycled with an initial denaturation at 96 °C for 10 mins followed by 40 cycles of 96 °C for 15 secs and 52 °C for 30 secs with data collection after each extension step.

Results and discussion

Cultivar resistance in U.K. OSR

The results from the 18 OSR varieties screened for resistance to *V. longisporum* demonstrated a range of susceptibility to the pathogen. Incidences ranged from an average of 28% in Palace to an average of 85% in PR45D05. No complete resistance was identified in any of the cultivars tested and all inoculated plots exhibited some level of disease symptoms. Though there was a large degree of variation between replicate scores from some cultivars, there were significant differences between cultivars ($p < 0.05$), as shown in Figure 1. Repeated tests across different sites and years are clearly needed to substantiate any potential sources of resistance identified within the material tested.

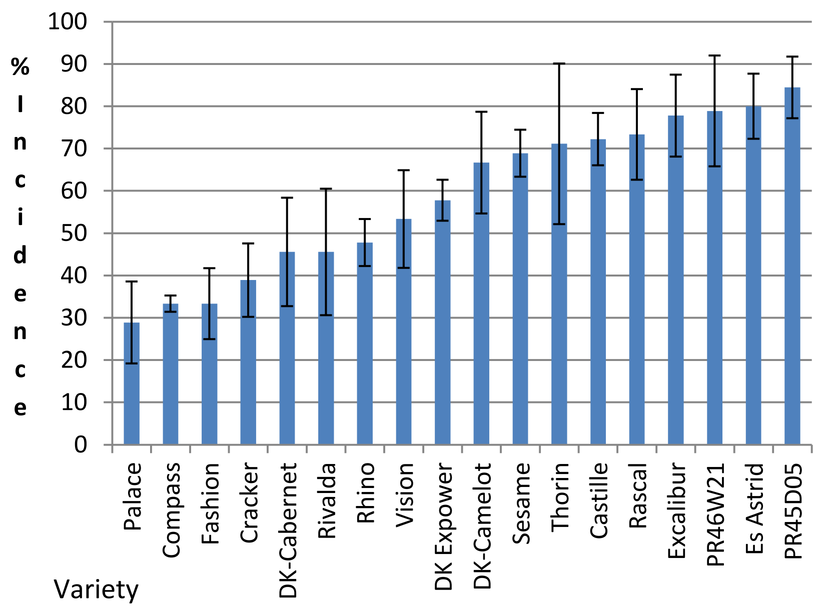

Figure 1. Histogram displaying *V. longisporum* disease incidences (%) in 18 OSR cultivars grown in inoculated field trials, NIAB HQ, Cambridge U.K., 2011-2012

Diagnostic assays

Three independent assays were developed to detect *V. longisporum* DNA extracted from soil. A CTAB and silicon dioxide pre-extraction and commercial kit extraction was employed to obtain qPCR quality DNA template from a series of spiked and naturally infested soil samples. DNA is lost during the extraction procedure so results from extracts have to be interpreted in semi-quantitative terms; differences between samples were observed between low, medium or high infestation samples. However, relative total DNA content can only be extrapolated by assessing the efficiency of the extraction process. This can be achieved by addition of a known quantity of DNA to a control soil sample run alongside the field samples.

LAMP, SYBR green and Taqman assays were designed around specific *V. longisporum* rRNA sequences. The LAMP assay was validated using genomic DNA dilution series as a template (20 ng - 2 fg) (data not shown). Positive reactions were recorded when an amplification peak was observed and the dissociation profile of each sample scrutinised to ensure only single amplification product was produced in each reaction. The assay was then used to screen soil DNA extracts from infested and control soil. Positive reactions were obtained from one of the three infested samples (and the control + DNA) (Table 1). The concentration of pathogen DNA in each of the samples was determined subsequently using qPCR. The two samples that failed to amplify contained approximately 2 fg and 10 fg DNA, respectively whereas the positive sample contained approximately 25 fg DNA in the samples tested. Although reliable when amplifying from larger amounts of template, the LAMP assay often failed to amplify samples containing a low amount of pathogen template.

Table 1. Positive/Negative results from DNA samples from inoculated plots (1-3), No Template Control (NTC), Control (CTRL) and Control + DNA (CTRL+) screened with LAMP assay

Sample	Result	Approx. DNA content (µl)
NTC	Negative	N/A
CTRL	Negative	N/A
CTRL +	Positive	100 pg/µl
1	Negative	2 fg/µl
2	Negative	10 fg/µl
3	Positive	25 fg/µl

A pair of SYBR green primers were designed and assessed for their performance to accurately detect *V. longisporum.* The assay performed optimally in the range of 2 ng/µl-2 fg/µl DNA and produced only a single amplification fragment (Figures 2 & 3). The assay was used to screen for pathogen DNA in artificially and naturally infested soil samples (Table 2). Clear differences in Ct values were detected between samples and DNA content increased proportionally in Low 3 fg/µl (< 50 MS), Medium 6 fg/µl (< 100 MS) and 20 fg/µl High (> 250 MS) spiked samples, respectively. The assay was able to detect DNA extracted from soil samples containing microsclerotia at a rate of approximately 5 or greater to 1-2 per gram of soil.

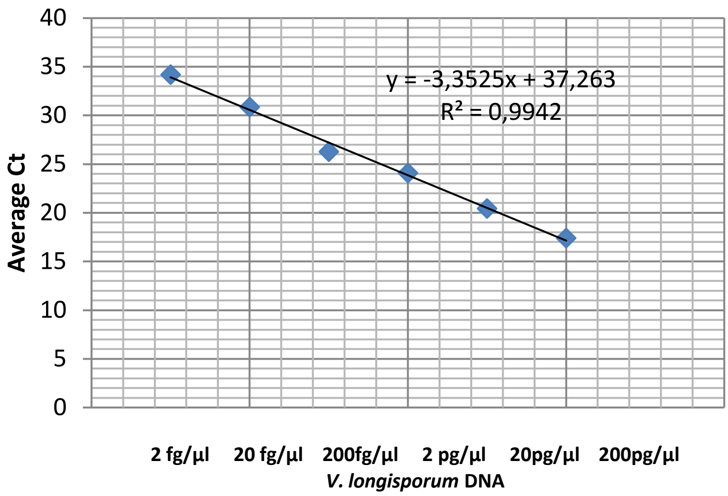

Figure 2. Standard curve generated using *V. longisporum* SYBR green primers (DNA concentrations 200 pg/µl-2 fg/µl)

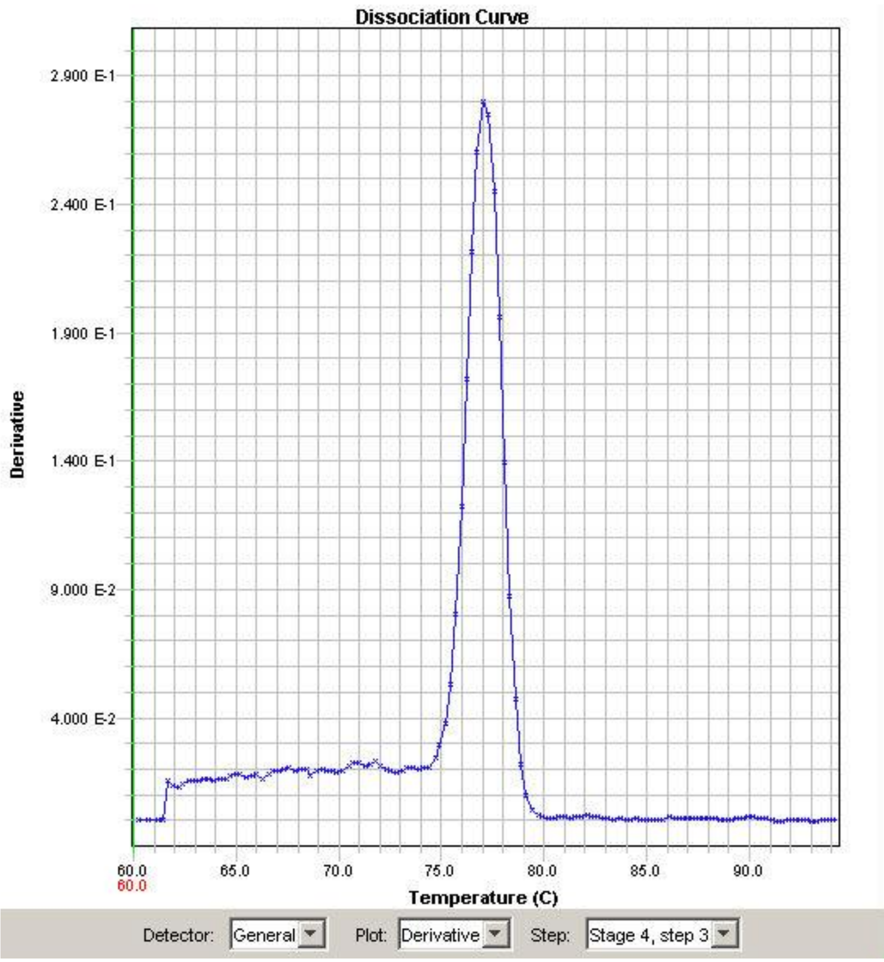

Figure 3: Dissociation curve generated using *V. longisporum* SYBR green primers run on soil sample extract

In samples from a moderately infested field-site the average estimated *V. longisporum* DNA content ranged from 2-25 fg/µl of extracted template (Table 3). This indicates the assay is detecting pathogen DNA within a range likely to be experienced in naturally occurring infestations. The average recovery using the DNA extraction procedure is approximately 5-10%. To assess the amount of DNA lost during the extraction this experiment, 200 ng of *V. longisporum* DNA was added to the control + sample during homogenisation. From the 200 ng DNA added, approximately 100 pg/µl (10 ng total) were obtained giving a 5% recovery rate during the extraction.

Table 2. Differences in Ct values in 50 g soil samples spiked with Low (< 50), Medium (< 100) and High (< 250) inoculum loads

Sample	Average Ct	DNA (µl)
NTC	0	0
CTRL	0	0
LOW (< 50)	33.87	3 fg/µl
MED (< 100)	32.67	6 fg/µl
HIGH (> 250)	30.57	20 fg/µl

Table 3. Differences in Ct values in soil samples from inoculated plots (1-3), No Template Control (NTC), Control (CTRL) and Control + DNA (CTRL+) screened with the SYBR green assay

Sample	Average Ct	DNA (µl)
NTC	0	0
CTRL	0	0
CTRL+	18.48	> 100 pg/µl
1	33.99	2 fg/µl
2	33.08	10 fg/µl
3	31.17	25 fg/µl

A Taqman probe and primers were designed to amplify and assessed for their performance to accurately detect *V. longisporum*. They were used to screen the naturally infested soil samples. The assay performed relatively accurately from 20 pg - 20 fg, although the amplification efficiency varied at different template concentration (Figure 4).

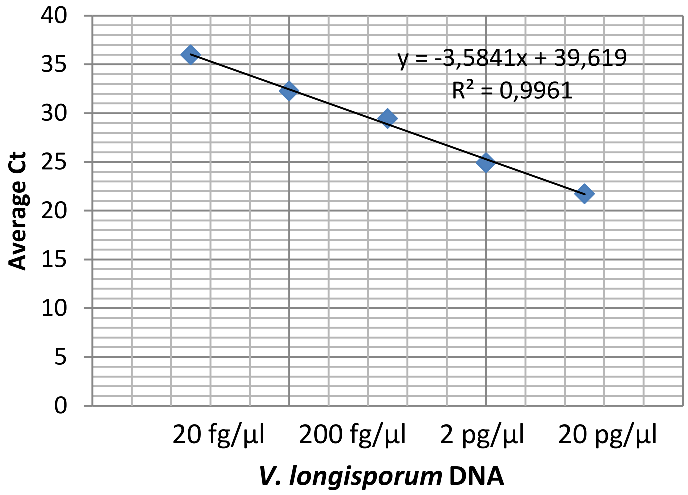

Figure 4. Standard curve generated using *V. longisporum* Taqman probe/primers (DNA concentrations (20 pg/µl-2 fg/µl)

Naturally infested soil samples were screened and although the Taqman assay could not accurately quantify DNA concentrations lower than 20 fg in samples, amplification was still observed in extracts with low DNA content (Table 4). In this instance the SYBR green assay appears to out-perform the Taqman assay. Continued development will enable more efficient Taqman probes/primers to be designed to detect *V. longisporum* quantitatively.

Table 4. Differences in Ct values in soil samples from inoculated plots (1-3), No Template Control (NTC), Control (CTRL) and Control + DNA (CTRL+) screened with the Taqman assay.

Sample	Average Ct	DNA (µl)
NTC	0	0
CTRL	0	0
CTRL+	22.066	> 100 pg/µl
1	38.115	< 20 fg/µl
2	39.0448	< 20 fg/µl
3	35.909	20 fg/µl

The identification of partial resistance to *V. longisporum* in some current OSR cultivars suggests a genetic component may be contributing to this variation. Further investigation into potential genetic resistance against the pathogen could help identify important resources thorough which to breed improved crop cultivars. Some newly released material claims resistance to *V. longisporum* and these are currently being evaluated in further inoculated field experiments.

The development of new diagnostic assays now enables pathogen DNA to be detected in a semi-quantitative manner, with a sensitivity of a single microsclerotium per gram of soil. The inclusion of specific marker DNA and corresponding detection assays to estimate DNA extraction efficiency could aid in accurate quantification of total target DNA content in infested soil. The techniques described could be utilised to investigate the impact of *V. longisporum* on OSR cultivation and such tools provide potential strategies to enable more rigorous testing of seed lots, screening of infested land and facilitate the development of risk management strategies to help growers to limit the impact of disease on crop productivity.

Acknowledgements

Funding from the BBSRC Research Experience Placement Programme and Syngenta Ltd. is gratefully acknowledged.

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IOBC-WPRS Bulletin Vol. 104, 2014
pp. 157-158

Cross-resistance in winter oilseed rape (*Brassica napus*) against multiple vascular pathogens

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Abstract: Oilseed rape (OSR) is one of the most important oilseed crops in the world. Due to its high yielding potential and a wide range of uses, the value of OSR has increased over time and currently it is intensively cultivated in many regions of the world. However, these situations created conditions for the emergence of economically important diseases including vascular pathogens. Due to the unique life style and means of survival, limited options are available to control vascular diseases and studies suggest that plant resistance, which provides a reasonably sustainable solution, remains the preferred strategy. In the present study the resistance reactions of two winter OSR cultivars previously identified as resistant and susceptible to *Verticillium longisporum*, were tested against *V. dahliae*, *Fusarium oxysporum* f. sp. *conglutinans* and *Xanthomonas campestris* pv. *campestris*. The experiment was conducted under greenhouse conditions. *V. longisporum*, *V. dahliae* and *F. oxysporum* f. sp. *conglutinans* inoculations were performed with a root-dip inoculation method. Infection with *X. campestris* pv. *campestris* was done by injecting bacterial cultures into major veins of young leaves. Disease development was assessed by evaluating disease severity, stunting effects, and reduction of plant biomass. Accumulation of the plant-defence related phytohormone salicylic acid (SA) was quantified by HPLC.

The results showed that unlike the *V. longisporum* susceptible cultivar Falcon, resistance of cultivar SEM to *V. longisporum* was verified as evidenced by significantly lower levels of net AUDPC, relative stunting, and dry matter yield reduction values. Interestingly, SEM also showed complete resistance towards *F. oxysporum* f. sp. *conglutinans* with mean disease index value of 0.78 compared to 6.56 in the *V. longisporum* susceptible cultivar Falcon. Seven days after inoculation, *F. oxysporum* f. sp. *conglutinans* significantly reduced (63%) dry matter (DM) yield in Falcon but had little effect on SEM and plants remained healthy even after 28 DPI with no significant difference between non-inoculated plants. In contrast, the rate of *F. oxysporum* f. sp. *conglutinans* disease development in susceptible plants was quick and caused complete death in less than 10 days whereas death took at least four weeks for *V. longisporum*. Both *V. longisporum* and *F. oxysporum* f. sp. *conglutinans* induced stunting and reduction of stem diameter in Falcon and SEM but the effects were more pronounced due to *V. longisporum* in the cultivar Falcon. In contrast to *V. longisporum* and *F. oxysporum* f. sp. *conglutinans*, infection with *V. dahliae* and *X. campestris* pv. *campestris* had no significant effect in all evaluated parameters. As expected, infection with *V. dahliae* did not induce disease in both genotypes confirming its non-pathogenicity to *B. napus*. Similarly, *X. campestris* pv. *campestris* infection did not cause distinct symptoms in both cultivars and infection was restricted only to infected leaves. This might be either due to less aggressiveness of the *X. campestris* pv. *campestris* strain or both genotypes might also be tolerant to this particular bacterial strain. Analysis of total SA (free and conjugated) accumulation in hypocotyl tissue revealed that irrespective of genotype, infection with *F. oxysporum* f. sp. *conglutinans* and *V. longisporum* induces significantly higher levels of SA. At 7 DPI, total SA accumulation in non-inoculated SEM and Falcon plants was 75 and

243 µM/g DW but infection increased the levels to 1.327 and 1.516 µM/g DW respectively. Similarly, at 28 DPI, *V. longisporum* induced up to a 4 and 10 fold increase in total SA levels in SEM and Falcon, respectively. In contrast, *V. dahliae* and *X. campestris* pv. *campestris* infection did not significantly alter accumulation of SA in both cultivars which is in agreement with the results obtained from the evaluation of disease parameters.

In conclusion, the resistance response of cultivar SEM to different vascular pathogens suggests that previously reported horizontal *V. longisporum* resistance mechanisms in *B. napus* (vascular occlusions, phenolics and lignin) might also effectively work against other aggressive pathogens that colonize the vascular system. The significantly higher accumulation of SA in infected resistant and susceptible cultivars and the very strong correlation found between disease levels and accumulation of SA due to *V. longisporum* (r^2 = 0.93) and *F. oxysporum* f. sp. *conglutinans* (r^2 = 0.87) indicated that SA played no significant role in conferring resistance in the pathosystem. Nevertheless, exploring the mechanisms behind cross-resistance of *B. napus* towards multiple vascular pathogens forms the focus of our future study.

Key words: cross-resistance, vascular pathogens, *Brassica napus, Verticillium longisporum, Fusarium oxysporum*

Clubroot

Integrated Control in Oilseed Crops
IOBC-WPRS Bulletin Vol. 104, 2014
pp. 161-168

Studies of clubroot (*Plasmodiophora brassicae*) on oilseed rape in the Czech Republic

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Abstract: Clubroot caused by the pathogen *Plasmodiophora brassicae* (Wor.) has been spreading on winter oilseed rape in Czech Republic for the last three years. Research on *P. brassicae* in Czech Republic is therefore important for the adoption of management strategies against the disease under Czech environmental conditions. Pilot experiments with clubroot resistant cultivars of winter rape started in 2012. Experiments were made in greenhouse and field conditions. For greenhouse experiments, soil samples were collected from contaminated fields. Seeds of oilseed rape varieties declared by seed companies as resistant together with susceptible controls (variety Rohan and chinese cabbage variety Granaat) were sown both into soil samples and in the field that was severely and homogeneously contaminated by *P. brassicae*. Incidence and severity of symptoms on roots were evaluated after eight weeks. Presence of *P. brassicae* in roots was tested by PCR. Results obtained from both experiments were almost identical. Resistant cultivars Mendel, Mendelson, Alister and Andromeda showed no visible symptoms in any soil sample, although PCR confirmed pathogen presence in all cases. Similarly, the soil samples were also tested for the presence of *P. brassicae* using PCR. Furthermore, testing of soil samples at different dilutions by RealTime-PCR using TaqMan probes and SYBR green is in progress. The monitoring of clubroot *P. brassicae* pathotypes diversity in the Czech Republic is also planned and has already started.

Key words: Clubroot, winter rape, *Plasmodiophora brassicae*, resistant cultivars, qPCR

Introduction

Winter oilseed rape is the second most important crop in the Czech Republic (Czech Statistical Office, 2013). Clubroot, caused by *Plasmodiophora brassicae*, was previously a problem in vegetable production, but the disease begins to appear on oilseed rape in recent years. Infected stands were reported throughout the country in fall 2011. Serious infestation was found on 44 farms, mainly in the north and north-east of the country (Czech Statistical Office, 2013). Although the pathogen appears to be widely spread across the whole country, its appearance in agricultural areas depends on appropriate weather conditions in the sowing period in August. Yield losses on rape aren't so high on national scale. It is probably because amounts of inoculum and distribution of pathogen within fields aren't sufficient. Nevertheless, there are fields which are already heavily infested. The situation is more serious, because there is not yet an easy, cheap and ecological method of crop protection against this pathogen. The problem could be solved by using resistant winter oilseed rape cultivars, but it is also necessary is using the right field crop rotation (Diederichsen, 2009; Hwang, 2012). Therefore, it is one part of research targeted on resistant cultivars. For good assessment of pathogen potential it is also necessary to know the inoculum amount – the spore load. *Plasmodiophora brassicae* has long living, hardy spores, which persist and remain viable in soil for up to 20 years (Wallenhammar *et al.*, 1996). For complex knowledge about clubroot it is also necessary to monitor pathotypes of *P. brassicea* in the Czech fields. In countries where monitoring has been done, results show highly diverse population containing up to 20 pathotypes with one or two prevalent pathotypes (Diederichsen *et al.*, 2009; Donald *et al.*, 2006; Strelkov *et al.*, 2007). Preliminary experiments with soils infested by *P. brassicae* have already been started in the Czech Republic and likewise the monitoring of clubroot pathotypes in the country is also in process.

Material and methods

Cultivar testing in the field

Five resistant cultivars, namely Mendel, Mendelson, Alister, Andromeda, and NV 2012 of winter oilseed rape were tested directly in *P. brassicae* infested field in the Hradek nad Nisou locality. Susceptible variety Rohan was used as a control. Cultivars were sown in three different time terms: 2nd August, 15th August and 4th September 2012. Every experiment was sampled after one month and plants with clubroot symptoms were monitored. The cultivars were then assessed on the basis of a clubroot rating scale: 0 – no clubs, 1 – small clubs (low infection), 2 – medium clubs (moderate infection) and 3 – big clubs (severe infection). All variants were also tested for the presence of *P. brassicae* by PCR.

Soil sample collection

Soil samples infested with *P. brassicae* were collected from 14 different fields in the Czech Republic (CZ) based on high yield loss in the previous year. Soil samples 1, 2, 3, 4, 5, 6, and 7 were from the north of CZ, samples 9 and 15 were from the center of CZ, samples 11 and 12 were from the southwest of CZ, sample 13 was from southeast of CZ, samples 14 and 16 were from the east of CZ.

Cultivar/soil testing in the greenhouse

In 2013, six resistant cultivars and one susceptible control cultivar were tested in above mentioned soil samples. Cultivars such as Mendel, Mendelson, CWH, RAP, Alister and X10W were each sown in pots with infested soil from certain fields. The clubs were monitored after 8 weeks and rated according to above mentioned clubroot rating scale. An index of disease (ID) was also calculated for each tested plant genotype, using the method of Horiuchi and Hori (1980) as modified by Strelkov *et al.* (2006):

$$ID = ((n \times 0 + n \times 1 + n \times 2 + n \times 3)/(N \times 3)) \times 100\%,$$

where N is the total number of plants, n is the number of plants in each class, and 0, 1, 2 and 3 are the symptom severity classes according to the clubroot rating scale.

Soil samples testing

Sufficient amount of each soil samples was collected for molecular analyses and DNA were subsequently isolated using GeneMATRIX Soil DNA purification kit (EURx, Gdansk, Poland). The PCR was then performed using the primer/probe described in Table 1.

The primers were designed using the “GenScript online PCR primer design tool” (http://www.genscript.com/). For the analysis, soil samples were diluted at different concentration 1:5, 1:10, 1:100, 1:1000, and 1:10000.

Table 1. Primer and probe used for the detection of *Plasmodiophora brassicae*

Primer/ probe name	Sequence	Reference
PbF	5′-AAACAACGAGTCAGCTTGAATGCTAGTGTG-3′	
PbR	5′-CTTTAGTTGTGTTTCGGCTAGGATGGTTCG-3′	Cao *et al*., 2007
PbF	5′-AACCATCCTAGCCGAAACAC-3′	
PbR	5′-ATTCTGCAATTCGCACTACG -3′	
probe PbP	5′-CGCAGTTCGCTGCGT TCTTCA-3′	

Pathotype testing

Preliminary tests to check the pathotypes according to ECD set (European clubroot differential set) obtained from Warwick Genetic Resources Unit (Warwick Crop Centre) are in process. They are based on 15 differential plant species and their varieties from *Brassiceae* family and moreover, susceptible winter oilseed rape cultivar Ladoga was also added. For initial testing, 5 localities from experiment with resistant cultivars were chosen in different soil (samples 4, 7, 9, 12, and 14). For inoculation, clubbed roots of control plants from the same experiment were used.

Results and discussion

Field performance of resistant cultivars

Experiments were planned with five cultivars claimed to be clubroot resistant (Mendel, Mendelson, Alister, NV 2012 and Andromeda). These cultivars were thus tested directly in *P. brassicae* infested fields. Cultivars were sown at three different time points: 2nd August, 15th August and 4th September 2012 as shown in Table 2. There were found significant differences between the reaction of resistant varieties and susceptible variety Rohan. Especially early sowing (2nd August) leads to big club formation in this cultivar. Later sown plants (15th August) had smaller clubs and plants sown at the last term (4th September) had almost no visible symptoms. Cultivars Mendel, Mendelson and Andromeda were without symptoms irrespectively of the term of sowing. Cultivar Alister slightly reacted when it is sown very early. Roots of NV 2012 were clubbed also later (15th August) as shown in Figure 1 (data shown only for Mendel). That fact is in correlation with higher temperatures at beginning of August, as higher temperatures support club formation (Gossen *et al.*, 2012). Concerning weather, year 2012 was favourable for clubroot development as both temperature and precipitation were high during August. Thus, very early sowing practiced by some farmers may be dangerous on infested fields if susceptible varieties would be used. Unfortunately observation of this experiment could not be prolonged further as the farmer needed this field for another crop.

Table 2. Effect of the sowing date on clubroot formation on different winter oilseed rape cultivars.

	Time point of sowing		
Cultivars	2nd August	15th August	4th September
Mendel (R)	0	0	0
Mendelson (R)	0	0	0
Alister (R)	1	0	0
Andromeda (R)	0	0	0
NV 2012 (R)	2	1	0
Rohan (S)	3	3	2
Legend: (R)/(S) – cultivar classified to be clubroot resistant/susceptible Rating values 0, 1, 2 and 3 represent formation of no, small, medium and big clubs.			

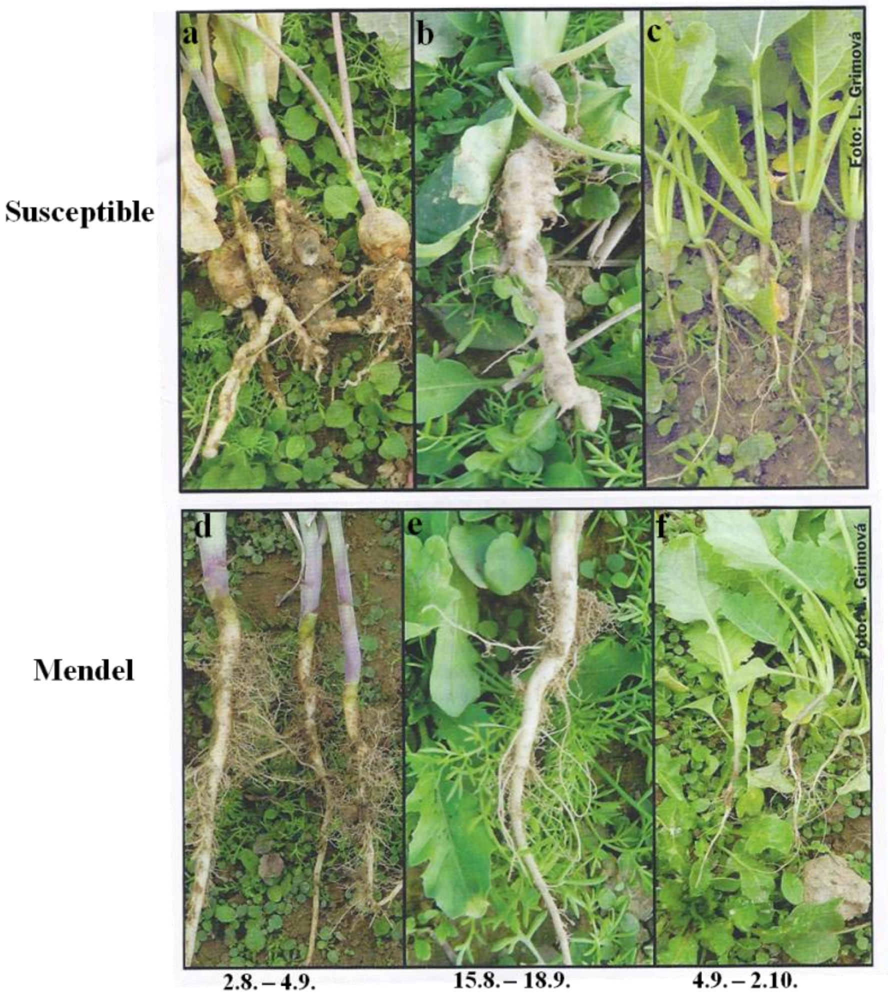

Figure 1. Effect of the sowing date on clubroot formation on the susceptible cultivar Rohan (a, b, c) and resistant cultivar Mendel (d, e, f) grown in three different time periods: 2^{nd} August - 4^{th} September (a, d) , 15^{th} August - 18^{th} September (b, e) and 4^{th} September - 2^{nd} October (c, f).

Cultivar performance in soil samples of different geographic origin

Six resistant cultivars, namely Mendel, Mendelson, CWH, RAP, Alister, and X10W, together with a susceptible control cultivar were tested in 14 *P. brassicae* infested soils from the Czech Republic. The aim of the experiment was to test whether resistant cultivars work in different soil types in combination with various *P. brassicae* pathotypes. Symptoms of clubroot were evaluated after 8 weeks on a clubroot rating scale of 0-3, as detailed above. Values were converted to an index of disease (ID) indicative for the relative amount of disease formation on all plants of each genotype tested. According to the low average (ID 2.4%) in soil sample 13, which was from a warmer region without sufficient precipitation, low levels of *P. brassicae* inoculum were found (Figure 2). These results supported the hypothesis that for infection, sufficient soil moisture was necessary, while in dry soils, spore formation was lower and likewise there was a lower inoculum level (Gossen, 2012). The north and the east part of CZ had higher precipitation and the temperature conditions were optimal for the formation of higher numbers of spores. This could be one of the reasons why plants growing in these soil samples were highly infested with *P. brassicae.* It was found that soil samples from the northern (1-7) and the eastern regions (14, 16) had significant levels of infection and this was reflected in the higher ID scores for all resistant and susceptible cultivars (Figure 2). However, spore formation doesn’t just depend on temperature and soil moisture. The different combinations of weather conditions vary throughout whole growing season of winter oilseed rape and differences between growing seasons are also important. All factors can generate different responses to infection in the same climatic region, which can be seen on samples from the centre of CZ (soil sample 9 and 15) and the southwest part of CZ (soil sample 11 and 12), where the ID was moderate for all resistant and susceptible cultivars.

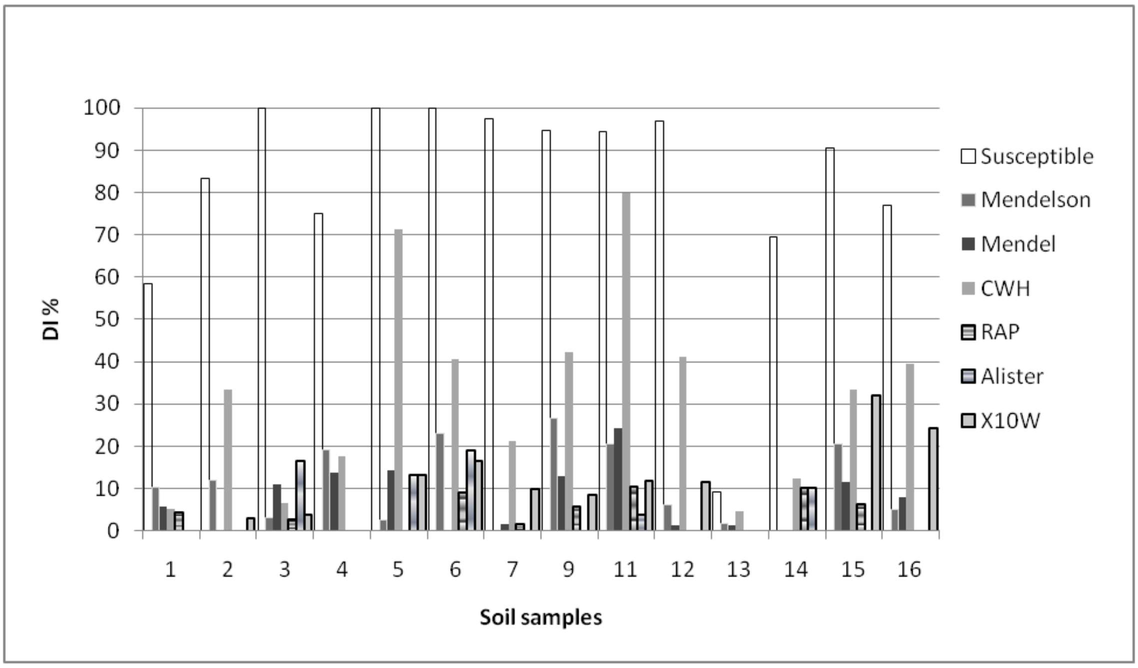

Figure 2. Index of clubroot disease (ID, %) of different winter oilseed rape cultivars tested in different soils samples (1-7, 9, 11-16) originated from different regions across the Czech Republic.

Resistant cultivars tested in greenhouse – comparison of cultivars

The same cultivars tested under field conditions were also tested in the greenhouse. The cultivar Alister was identified as the most resistant cultivar in response to soil samples from various geographical locations. Alister showed total resistance to nine samples and ID's were 0, although average ID over all samples was 4.5%, which is slightly higher than for cultivar RAP (3.6%). Alister became infected in five soil samples, with the highest ID (19%) for the soil sample 6 from the north part of CZ and the lowest ID for sample 11 (3.7%) from the southeast. Also, for soil sample 3, from the north of CZ, Alister was the most infected of the resistant cultivars (Table 2). These data support the above mentioned result that the soil samples from the north part of CZ (sample 1-7) had a high level of inoculum and the chances of occurrence of infection from *P. brassicae* were high. The resistant cultivars RAP and X10W had also good performance in resistance; averages over all samples were 3.6% and 9.6%, respectively. These cultivars had also ID of 0% in sample 13.

Cultivar CWH claimed by the breeder as resistant was found to have significantly higher infection level for all soil samples. In this cultivar the highest ID was found for soil sample 11 from the southwest part of CZ (80%) and the lowest ID for sample 1 from north (5.1%). It was interesting to note that some plants of this cultivar were totally clubbed and some plants were totally healthy, so it seemed that some plants were resistant and some were not.

For resistant cultivars Mendel and its descendant Mendelson, significantly high ID (24.2% and 20.5%, respectively) were found for the soil sample 11 from the southwest part of CZ, as compared to lower levels of infection for soil sample 13 from the southeast (1.45% and 1.67%). The results demonstrated the infection of these cultivars in high and low infested soil samples. These cultivars brought an average performance in resistance among all soil samples. The reason could be due to unique mixture of pathotypes of *P. brassicae* in each soil sample. These resistant cultivars were bred against most prevalent European pathotypes, but this means that the resistance doesn't cover every pathotype in Europe (Diederichsen, 2009) (Table 3). This situation could later be explained by pathotype determination. Maybe also the level of inoculum could complicate the situation. Perhaps some varieties could resist only low level of infestation, but higher levels of infestation could overcome the resistance.

Table 3. Index of clubroot disease (ID, %) of different winter oilseed rape cultivars grown in different soil samples originated from different regions of the Czech Republic.

Soil sample	1	2	3	4	5	6	7	9	11	12	13	14	15	16	Mean
Rohan	58.3	83.3	100.0	75.0	100.0	100.0	97.4	94.6	94.4	97.0	9.26	69.4	90.7	76.9	81.9
Mendelson	10.4	12.0	3.0	19.0	2.6	23.1	0	26.7	20.5	6.1	1.67	0	20.4	4.9	10.7
Mendel	5.9	0	11.1	13.7	14.3	0	1.5	13.0	24.2	1.5	1.5	0	11.6	8.0	7.6
CWH	5.1	33.3	6.7	17.6	71.4	40.7	21.2	42.2	80.0	41.3	4.8	12.5	33.3	39.6	32.1
RAP	4.5	0	2.8	0	0	9.1	1.5	5.8	10.4	0	0	10.3	6.41	0	3.6
Alister	0	0	16.7	0	13.3	19.0	0	0	3.7	0	0	10.3	0	0	4.5
X10W	0	2.9	3.7	0	13.3	16.7	10	8.6	11.8	11.6	0	0	32.1	24.4	9.6
Mean	12.0	18.8	20.6	17.9	30.7	29.8	18.8	27.3	35.0	22.5	2.4	14.6	27.8	22.0	

Testing soil samples for contamination with* P. brassicae *by the use of PCR
We have also tested 14 different soil samples infested with *P. brassicae* from different fields in the Czech Republic using conventional PCR. We found the presence of *P. brassicae* in all samples (Figure 3). Further, we have tested 2 soil samples diluted by non-infested soil in ratio 1:5, 1:10, 1:100, 1:1000, and 1:10000 using qPCR.

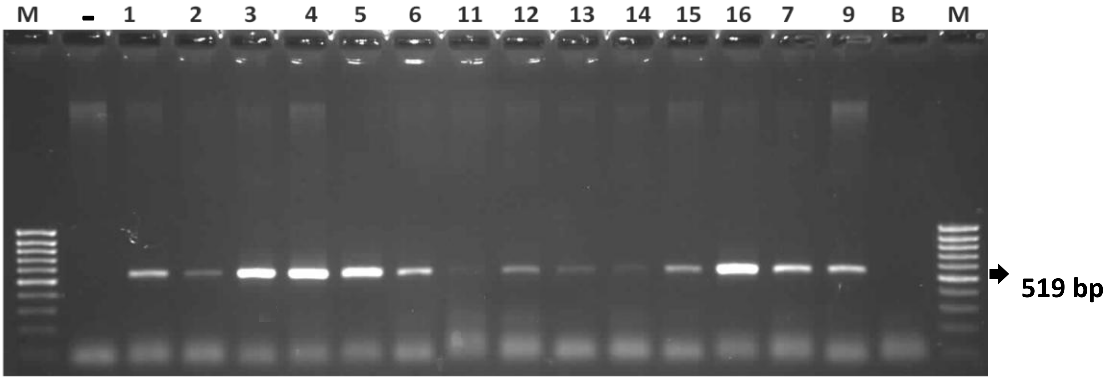

Figure 3. PCR test for the presence of *Plasmodiophora brassicae* in different soil samples collected from different regions across the Czech Republic.
M (Marker), „-" (soil without *Plasmodiophora brassicae*), 1-16 (soil samples naturally infested with *P. brassicae*) and B (Blank). Amplicon size: 519 bp.

Pathotype testing
The initial testings have delivered some interesting results. Three of five tested soil samples could be counted in testing. Samples 4, 7 and 14 had significant reactions. Successful inoculation by *P. brassicae* was proved by infection of control ECD 05. This is variety of Chinese cabbage cv. Granaat, which is susceptible to all pathotypes. According to the reaction of the ECD differential set, soil sample 4 matched to pathotype ECD 16/712, sample 7 to ECD 16/14/12 and sample 14 to ECD 16/3/28. ECD 05 displayed low reaction in soil samples 9 and 12 and couldn't be counted in testing. ECD 16/3/28 and ECD 16/14/12 have already been found in previous tests (Rod, 1994), which were done on vegetable brassicas and correspond to pathotype 6 and 7+4, respectively, on the Williams's differential set (Williams, 1966). All tests have to be repeated for confirmation of the results.

Acknowledgement

This work was supported by the Czech Ministry of Agriculture (grant QJ 1310227). We are grateful to Czech Union of Growers and Processors of Oilseed (SPZO) for providing soil samples.

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IOBC-WPRS Bulletin Vol. 104, 2014
p. 169

The importance of post harvest soil management in oilseed rape fields in reduction of clubroot severity

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Abstract: *Plasmodiophora brassicae* Woronin is an obligate biotrophic pathogen with a wide host range of 300 species of cruciferous plants and is an emerging threat to German oilseed rape (*Brassica napus*) production. To date, few commercially acceptable resistant cultivars are available and no fungicides are currently registered for the control of this pathogen on OSR in Germany. Previous studies indicated that oilseed rape volunteers and weeds play a critical role in predisposing of disease incidence and severity. A series of studies were conducted under greenhouse conditions with a susceptible OSR cultivar to clubroot disease to assay the effect of foliar application of the herbicide glyphosate and mechanical destruction of OSR volunteers in reducing clubroot disease severity. Plants were inoculated by injecting spore suspension (2 x 10^7 spores/ ml) beside root hairs at BBCH 13-14. To determine the effect of timing of applications, plants were treated early (seven days after inoculation, dpi) or late (21 dpi). Plants were examined for clubroot development 35 days after inoculation. The roots were dug from the soil, washed with the tap water and assessed for the severity of gall formation. Visual disease assessments after early and late applications showed variation among the treatments in symptom development. Changing the time of application had a significant effect on control efficiency. Results from this study demonstrated that the early application of glyphosate as well as the early mechanical destruction significantly decreased, relative to untreated control, the development of clubroot symptoms on infected roots. In particular, early glyphosate treatment reduced disease incidence and severity in treated plants. The results suggest the herbicide affected the development of the pathogen's host plant which had the effect of weakening the obligate pathogen. An early application of glyphosate could potentially interrupt the pathogen’s development and suppress the establishment and survival of the resting spores.

Key words: clubroot; control measures, timing, efficacy

Integrated Control in Oilseed Crops
IOBC-WPRS Bulletin Vol. 104, 2014
pp. 171-175

Infestation of Polish agricultural soils by *Plasmodiophora brassicae* on the Polish-Belarussian border in Podlasie province

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Abstract: Extensive cultivation of oilseed rape worldwide, leads to tight rotations in regions suitable for the production of this cash crop. Growing of the same plant species on large areas and with only short breaks of crops having different requirements lead to accumulation of pests and diseases. In recent years in Poland a great increase of soil infestation by the soil-borne protist-like pathogen *Plasmodiophora brassicae* has been observed. This is why we have undertaken a methodical survey of agricultural soils in the whole country. In this paper we present the results of investigations done in 2012 and 2013 in Podlasie province (Podlaskie voivodeship) in counties located along the Polish-Belorussian border. The study was done in six counties, including one municipal county of Bialystok (1 sample) and five rural counties: Augustowski (7), Bialostocki (18), Hajnowski (8), Siemiatycki (9) and Sokolski (7). Apart from one sample from the field located in a municipal area, the samples were collected from 49 fields, 28 of which were located in typically rural communities and 21 originated from urban-rural communities. Sampling was done using a soil auger. Depending on field size, a sample was taken from 8 to 10 locations per field, 200 g of soil per location. In total *ca.* 1.6 to 2 kg of soil was collected per field. The detection of the presence of *P. brassicae* was done using a soil test performed in glasshouse conditions at 20 °C, using seedlings of two species of the genus *Brassica*, susceptible to *P. brassicae*. Eight weeks after sowing the plants were individually removed from the soil samples and were inspected for the presence on clubs on roots. The pathogen was found in 3 out of 50 fields monitored (6%), with one sample per Bialostocki, Hajnowski and Sokolski county. The pathogen was not found in samples from the field located in Bialystok municipal county. We conclude that the serious occurrence of *P. brassicae* is occasionally present in agricultural soils in Podlasie province in counties along the Polish-Belarussian border.

Key words: clubroot, *Plasmodiophora brassicae*, soil-borne disease, soil auger, winter oilseed rape

Introduction

Clubroot – the disease of oilseed rape, vegetable brassicas and cruciferous weeds – is caused by *Plasmodiophora brassicae* Woronin. The pathogen is a soilborne, obligate biotroph with a complex life cycle consisting of two main phases, the first occurring in root hairs and the second present in cells of the root cortex (Mithen & Magrath, 1992). The infection of plants manifests itself in the formation of profound clubs that may completely destroy roots, thus inhibiting the uptake of water and nutrients. The plants with roots transformed to galls die quickly especially if free water is scarce. Resting spores of *P. brassicae* can survive in soils for a long time and they are also present in pond water sediment (Datnof *et al*., 1984). The use of such water is likely to spread the pathogen into pathogen-free seedbeds and fields. Cultural practices such as liming and the use of trap plants, although very useful (Yamagishi *et al*., 1986; Cheah *et al*., 2006; Bhattacharya & Dixon, 2008; Robak & Gidelska, 2013) are insufficient to completely control the disease. Chemical treatment has also been unsuccessful in protecting crops often proving to be too expensive. The most relevant and economical way of disease control is the development and growth of resistant cultivars (Manzanares-Dauleux *et al*., 2001).

The disease has a high economic impact (Dixon *et al*., 2009). It commonly occurs in all *Brassica*-growing areas in Europe and North America (Pageau *et al*., 2006; Diederichsen *et al*., 2009). For the last few years the pathogen poses a serious challenge to growers of oilseed rape in Poland where disease was reported by farmers from different regions of the country (Jedryczka *et al*., 2013). In Poland, a project devoted to the study of the incidence of clubroot on oilseed rape and the search for sources of genetic resistance of interspecific hybrids and mutants of *Brassica* was initiated in 2010. The aims of the project are: i) to recognize the composition of the population of *P. brassicae* in Poland; ii) to search for sources of genetic resistance to clubroot; iii) to study the usefulness of molecular markers of resistance to clubroot; iii) to study physical location of resistance to clubroot in the genomes of interspecific hybrids. The work presented here is a part of the project. Our aim was to examine the incidence of *P. brassicae* in agricultural soils in north-east Poland, along the border with Belarus.

Material and methods

Soil samples were collected randomly from fields with agricultural crops. The monitoring was done in 2012 and 2013, in counties located in Podlasie province, located along the Polish-Belarussian border, as shown in Figure 1.

Figure 1. A map of Poland with marked studied region (rectangle box) showing the Polish-Belarussian border (dotted line) located in Podlasie province.

In total 6 counties were monitored, with 5 rural and 1 municipal county (Bialystok). The fields in rural countries were located in typical rural communities (Powiaty) (28 samples), and in urban-rural communities (21 samples). Out of 49 fields located in rural or semi-rural communities, 7 fields were located in Powiat (county) Augustowski, 18 fields in Bialostocki county, 8 fields in Hajnowski county, 9 in Siemiatycki county and 7 fields were located in Sokolski county (Table 1).

Table 1. Infestation by *Plasmodiophora brassicae* of soil samples collected at random on the Polish-Belarussian border in Podlasie province in 2012 and 2013

No.	Field location	Coordinates		County (Powiat)	Type of community*	Biotest result	
		Latitude N	Longitude E			*B. rapa*	*B. napus*
1	Augustow 1	53 55 53,9	22 50 07,9	Augustowski	UR	0	0
2	Augustow 2				UR	0	0
3	Sztabin 1	53 46 19,7	23 14 52,1		R	0	0
4	Sztabin 2	53 44 45,1	23 15 07,5		R	0	0
5	Sztabin 3	53 43 24,1	23 11 55,7		R	0	0
6	Sztabin 4	53 42 38,0	23 09 16,4		R	0	0
7	Barglow Koscielny	53 46 18,0	22 49 09,8		R	0	0
8	Sokolka	53 24 06,4	23 29 26,7	Sokolski	UR	0	0
9	Suchowola	53 34 39,0	23 06 09,3		UR	0	0
10	Krynki	53 15 51,1	23 46 13,1		UR	0	0
11	Janow	53 27 25,4	23 13 40,8		R	0	0
12	Szudzialowo	53 17 53,9	23 39 13,1		R	0	0
13	Bialousy	53 24 39,2	23 13 04,1		R	0	0
14	Trofimowka	53 29 13,0	23 13 00,6		R	2.7	1.5
15	Choroszcz	53 09 10,0	23 02 04,4	Bialostocki	UR	3.6	3.1
16	Czarna Bialostocka	53 16 26,2	23 15 13,6		UR	0	0
17	Dobrzyniewo Duze	53 12 11,9	23 00 45,0		R	0	0
18	Grodek	53 09 31,3	23 32 27,2		R	0	0
19	Juchnowiec Koscielny	53 04 39,8	23 08 03,5		R	0	0
20	Lapy 1	52 59 15,6	22 52 58,5		UR	0	0
21	Lapy 2				UR	0	0
22	Michalowo	53 02 12,4	23 36 02,8		UR	0	0
23	Poswietne	52 56 10,0	22 51 20,9		R	0	0
24	Suprasl	53 11 00,0	23 18 00,0		UR	0	0
25	Suraz	52 56 59,5	22 57 06,6		UR	0	0
26	Turosn Koscielna 1	53 02 39,5	23 02 28,5		R	0	0
27	Turosn Koscielna 2	53 01 07,2	23 00 06,3		R	0	0
28	Tykocin	53 09 40,0	22 44 39,7		UR	0	0
29	Wasilow	53 11 24,3	23 14 07,0		UR	0	0
30	Zabludow	52 56 11,1	23 15 41,9		UR	0	0
31	Zawady	53 10 10,8	22 39 15,2		R	0	0
32	Dobrzyniewo Duze	53 07 56,3	23 10 07,4		R	0	0
33	Bialystok	53 12 11,9	23 00 45,0	Bialystok	M	0	0
34	Narew	52 56 22,2	23 26 20,5	Hajnowski	R	0	0
35	Narew	52 53 55,1	23 32 14,6		R	0	0
36	Białowieża	52 42 06,8	23 51 42,1		R	0	0
37	Hajnówka 1	52 44 27,7	23 34 41,6		UR	0	0
38	Hajnówka 2	52 45 00,0	23 30 00,0		UR	0	0
39	Czyze	52 44 38,1	23 25 39,2		R	3.4	2.9
40	Kleszczele	52 34 29,0	23 19 37,9		UR	0	0
41	Czeremcha	52 31 44,4	23 18 36,7		R	0	0
42	Milejczyce 1	52 30 27,9	23 13 21,6	Siemiatycki	R	0	0
43	Milejczyce 2	52 31 10,5	23 07 55,0		R	0	0
44	Nurzec Stacja 1	52 28 15,4	23 04 40,0		R	0	0
45	Nurzec Stacja 2	52 27 59,7	23 02 56,0		R	0	0
46	Mielnik	52 24 57,5	22 59 03,0		R	0	0
47	Siemiatycze	52 23 15,2	22 54 33,0		UR	0	0
48	Drohiczyn 1	52 24 01,3	22 39 23,2		UR	0	0
49	Drohiczyn 2	52 25 13,5	22 43 50,1		UR	0	0
50	Dziadkowice	52 33 51,1	22 54 39,7		R	0	0

* R – rural, UR – urban-rural, M – municipal

Soil sampling was done using a soil auger (Spychalski and Kosiada, model 117084, Agroekspert Polska). The soil was sampled from the layer 0-20 cm in depth. Depending on field size, a sample was taken from 8 to 10 locations per field, 200 g of soil per location. In total *ca.* 1,6 to 2 kg of soil was collected per field and mixed well with peat of pH 3.5 (2:1).

The biotest to detect the presence of *P. brassicae* was done under greenhouse conditions using the seedlings of susceptible accession of *Brassica rapa* ssp. *pekinensis* and a Polish variety of oilseed rape (*B. napus* cv. Monolit). Eight weeks after sowing, the plants were individually pulled off the tested soil and inspected for the presence on clubs on roots. The county was regarded as free from serious occurrence of the pathogen when none of the studied samples contained plants with visible clubs on roots.

Results and discussion

Most of the studied soil samples were free from the amounts of resting spores that could cause clubs on roots of susceptible plants. Out of 50 studied samples, 47 were free from severe occurrence of *P. brassicae* (Table 1). However, 3 samples – each originating from a different county – were positive to *P. brassicae*; two of these samples originated from rural communities (Czyze from Hajnowski county and Trofimowka from Sokolski county) and one from an urban-rural community (Choroszcz, Bialostocki county). Thus, the pathogen was found in 6% of monitored fields, located in 3 different counties along the border with Belarus, in Podlasie region. Together with samples studied more recently (data not presented) the positive signals of the presence of *P. brassicae* in this region was obtained from 4 out of 8 counties. The pathogen was not found in the field located in Bialystok municipal county. Based on these results we conclude that the serious occurrence of *P. brassicae* is occasionally present in agricultural soils in Podlasie province in counties along the Polish-Belarussian borderline. Further studies using molecular techniques may reveal the presence of the pathogen in some of the other samples collected in this area, however the level of their infestation would be – in most cases – insufficient to cause the disease symptoms in field conditions. The biotest used in this study greatly enhances the development of the disease, due to the use of susceptible plant genotypes as well as creating the conditions supporting the development of clubroot (wet, acidified soil, rich in nutrients).

In contrast to studies based on monitoring the disease in plants, this study aimed at monitoring of the levels of clubroot pathogen in agricultural soils taken from crop fields other than winter or spring oilseed rape. A similar study was performed in 2011-2012 in Wielkopolska province, located in central-west Poland (Jedryczka *et al*., 2013). In this study soil samples were obtained from 196 fields, encompassing 31 rural and 4 urban counties. Similarly to this study, the pathogen was not detected in any soil of municipal counties, but it was found in eight rural counties (25.8%). Random sampling of Polish agricultural soils on Polish-Ukrainian border also detected samples positive to clubroot (Jedryczka *et al*., 2014). Soil infested with *P. brassicae* was found in two out of nine rural counties; both of them were located in Podkarpackie Voivodeship, close to an international highway. This is why the actions of a preventive character should be undertaken, to significantly reduce the further spread of this dangerous pathogen.

Acknowledgements

This work was supported by the National Science Centre in Poland, project N N310 298439.

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IOBC-WPRS Bulletin Vol. 104, 2014
p. 176

Identification of the chromosome complement and genome recombination in interspecific hybrids and mutants within the genus *Brassica*, with known resistance to clubroot

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Abstract: Amphidiploid rapeseed is a very important oil plant that has become a widely cultivated crop in many countries worldwide. Searching for forms with improved traits is highly desirable and from that point of view, interspecific crossing is a valuable tool for widening the variability of useful traits, e.g. seed quality and resistance to some diseases such as clubroot (caused by the soil-inhabiting protist *Plasmodiophora brassicae*), which causes severe damage to oilseed rape and vegetable brassicas. The main sources of resistance used to date originate from different species of the genus *Brassica*, including *B. campestris* (A-genome), *B. oleracea* (C-genome) and *B. napus* (AC-genome). Different experimental approaches have been applied to study chromosome rearrangements in *Brassica* allotetraploid and ancestral genomes, such as the production of synthetic allopolyploids compared to natural forms using chromosome mapping and cytogenetic analysis including fluorescence and genomic *in situ* hybridization (FISH/GISH). Physical mapping of 5S and 18S-5.8S-26S (35S) rRNA genes by FISH provides valuable chromosomal landmarks, and their characteristic positions enable chromosome identification, which allows detection of chromosome variability. In this ongoing research, we focus our attention on (i) an analysis of ribosomal DNA (rDNA) loci number and location in individuals of F_3-F_6 generations, which resulted from the interspecific crosses between *B. napus* and *B. campestris* ssp. *pekinensis* as well as *B. campestris* ssp. *trilocularis*, (ii) determination of the parental genomes using FISH with C-genome specific BAC-based probes (BAC-FISH) and (iii) an assignment of known *Brasssica* chromosomal markers to their corresponding genomes in *Brassica* forms studied. Among *Brassica* interspecific hybrids, different number of both kinds of rDNA sequences were observed, indicating genome re-organization. The use of *B. oleracea* BAC clones revealed the chromosome re-arrangements between A- and C-genomes in the synthetic *B. napus* forms, which can be a rapid response to formation of the allotetraploid *B. napus* genome. The resistance of the different plant genotypes was studied using a biotest performed in controlled environment conditions. The seeds of hybrids, their parental lines and the mutants of *B. napus* were germinated for 5 days in petri dishes, then the small seedlings were transplanted to a soil substrate and inoculated with spore suspensions of different isolates/races of *P. brassicae*. Susceptibility/resistance of particular lines was assessed 8 weeks after plant inoculation and interpreted based on the results of cytogenetic studies. In this work, the identification of chromosome identity and their re-arrangements in synthetic *B. napus* forms will be presented.

Key words: clubroot resistance, oilseed rape, ancestors, allopolyploids, chromosome rearrangements

Acknowledgement

This work was supported by the National Science Centre, project N N310 298439.