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at

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Preface

Cultivation of oilseed crops has vastly increased in prominence over the past 5-10 years. Oilseeds now provide ca 12% of the global supply of edible oils (Zhu *et al.*, 2016 New Phytologist 210: 1169-1189); *Brassica napus* in particular, is now the world's third largest source of vegetable oil (http://faostat3.fao.org), which is used not only for human consumption but also as a valuable high-protein animal feed. In addition, it is also an important source of industrial lubricants and biofuel. The crop has risen in value such that it is now grown wherever and whenever possible, not simply as a break crop. However, EU capping on biofuels has unstabilized the market to some extent and reliance on heavy inputs, in particular fungicides and insecticides have meant that the crop returns much smaller margins than in recent years. Pest resistance is now a big problem; few new active ingredients have come to the market place and those that remain are becoming increasingly regulated. Farmers are running out of options for control in some cases. In the meantime, the FAO has called for the 'sustainable Intensification of agriculture'. To meet these aims and to provide growers with solutions it is clear that Integrated Pest Management techniques will be more important than ever.

The IOBC Working Group on Integrated Control in Oilseeds will meet for the 16th time in Tartu, Estonia, September 7-9, 2016, to address the *''Prospects and progress for sustainable oilseed crop protection''*. The meeting will be carried out in entomology and plant pathology subgroups but also we will have joint sessions where we can share knowledge through fruitful discussions. We will discuss the limitations and problems of chemical control including resistance, how technology – especially decision support can aid judicious use of pesticides, present preliminary findings of work done to develop new alternative methods of control, including biological control, breeding for resistance and agronomic techniques.

We are delighted that over 60 participants from 10 countries within Europe will attend this meeting! We are a *working group*, not merely a conference so we hope there will be many fruitful discussions leading to improved co-operations to address the big challenges in this area.

Welcome to Estonia! *Tere tulemast Eestisse*! We wish you a happy and productive meeting!

Malgosia ''Gosia'' Jedryczka, WG Convenor,
Sam Cook, Working Group sub-convenor and
Eve Veromann, Local Organizer

Sponsors

We would like to thank our sponsors:

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Estonian Society of Plant Protection

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Impact of different insecticides sprayed in autumn or treated to winter oilseed rape seeds on the number of *Psylliodes chrysocephala* larvae and TuYV infection

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Abstract: The influence of different insecticides applied to winter oilseed rape in autumn on cabbage stem flea beetles, aphids and virus infestation was investigated. In 2015 the effects of the seed treatments Elado and Fortenza Force on larvae of cabbage stem flee beetle were very limited, but adult beetles migrated into the field very late. Spraying of a pyrethroid (Karate Zeon) in autumn reduced the number of larvae by up to 80%. The aphid and virus infestation were significantly reduced only by using Elado as seed treatment.

Key words: cabbage stem flea beetle, TuYV, aphid, insecticides, seed treatment, spraying

Introduction

The cabbage stem flea beetle *Psylliodes chrysocephala* L. (Coleoptera: Chrysomelidae), is one of the most important autumn-occuring pests of winter oilseed rape *Brassica napus* L. (Alford *et al*., 2007). The abundance and damage potential vary between years and are dependent on temperature (Hoßfeld, 1993; Mathiasen, 2015). An important disease of winter oilseed rape is the turnip yellows virus (TuYV) (Stevens *et al*., 2008). The main vector of this virus is the green peach aphid *Mycus persicae* (Schliephake *et al*., 2000). Mild autumn and winter conditions can cause a high abundance of aphid vectors and encourage virus spread (Stevens *et al*., 2008). The control of these pests by insecticides (spraying and seed treatment) is an important and widespread effective tool for generating high yields (Nilsson, 2002, Alford *et al*., 2003). The influence of different seed treatments and spraying against winter oilseed rape pests (*P*. *chrysocephala* larvae, aphids) and the virus infection was investigated in this study.

Material and methods

The field experiment was conducted in a winter oilseed rape field (cv. Avatar) in Lower Saxony near Braunschweig (sown 26.08.2015). The trial area was divided into 16 randomized plots (each 0.072 ha) in a block design and subjected to the following treatments:
- untreated control;
- Karate Zeon (pyrethroid, lambda-cyhalothrin 7.5 g/ha, sprayed: 05.10.2015, BBCH 15);
- Elado (neonicotinoid + pyrethroid, clothianidin + beta-cyfluthrin, 10 + 2 g ai/kg);
- Fortenza Force (diamide + pyrethroid, cyantraniliprol + tefluthrin, 39 + 25 g ai/1.000.000 seeds).

The migration of adult *P. chrysocephala* into the field was monitored using yellow water traps which were sampled weekly. Plant damage caused by the adults was assessed on 10.09.2015, 17.09.2015, 24.09.2015 and 01.10.2015 by determining the % affected leaf area. Larval infestation of at least 10 plants were carried out weekly until first larval hatch was detected. To estimate larval infestation of plants, three plant samples were taken (30.11.2015; 15.02.2016, 15.03.2016). On each sampling occasion 20 plants were collected from each plot and manually dissected with the aid of a binocular microscope and the number of larvae was recorded. The BBCH of the crop was determined at each assessment day on the field.

Aphids were sampled on two occasions (30.09.2015 and 12.10.2015) and the 2 species *Myzus persicae* and *Brevicoryne brassicae* occurring were separated. The upper and underside of the leaves of 20 plants randomly selected per plot were assessed. To estimate the TuYV infection rate, the second oldest leaf from 12 randomly selected plants per plot were sampled on two occasions (10.12.2015 and 14.03.2016). A 50 mg sample of each leaf was stored in extraction buffer at -20 °C. Virus titer was determined using polyclonal TuYV specific antibodies from the Serum bank of JK and by DAS-ELISA according to Clark and Adams (1977).

Results and discussion

The main migration period of cabbage stem flea beetle into the field was between 22.09.15 and 12.10.15, BBCH 12-14 – BBCH 15-16. Karate Zeon was sprayed at the 12th of October when the threshold of 50 beetles per yellow water trap was reached. The feeding damage of the adults did not exceed 1.5% of the leaf area on average. A slightly higher but still negligible feeding damage was found in the control. This low damage level might have been caused by the late migration of the beetles.

First larvae were detected at the beginning of November 2015. On first sampling occasion for larvae on 31.11.15, small differences were found between the number of larvae per plant between treated plots and the control (Figure 1). By this date, only a small number of larvae had hatched also in control plots. During the winter period, the number of larvae increased up to 10 per plant. On the 2nd sampling occasion (15.02.16) the Karate Zeon treatment showed the lowest number of larvae per plant (about 2). In seed treated plots, there were only 1.5 (Elado) and 0.4 (Fortenza Force) fewer larvae per plant than in control plots (about 9). On the 3rd sampling occasion (15.03.16) again, the number of larvae were only significantly fewer than the control in the Karate treated plots (reduced by 5.2).

The late hatching of the larvae was induced by the cold conditions in September and October and the late immigration of the beetles. The insignificant effects of the two seed treatments may have been due to the late immigration of the beetles, which meant that plants were larger plants by the time immigration started and therefore lower concentrations of the active substances would have been present in the leaf tissue. Karate Zeon sprayed at the peak of immigration showed high efficiency on *P. chrysocephala*.

There was no clear reduction in numbers of both aphid species (Figure 2) in Karate Zeon (treatment at 5.10.15) and Fortenza Force seed-treated plots. For Karate Zeon it is unlikely that high effects would have occurred because exposure for aphids on the upper side of leaves is quite low. In Elado seed-treated plots, the number of aphids was clearly reduced. *M. persicae* is the most important vector of TuYV but also *B. brassicae* is relevant (Schliephake *et al*., 2000).The Elado seed treatment therefore offers the potential of protecting the crop against TuYV by reducing the number of potential vectors. This was supported by lower virus titers in leaves from Elado seed-treated plots, especially in the March sample

(Figure 3). Stevens *et al*. (2008) also documented a reduction of virus infection by clothiandin seed treatment. The percentage of infected plants of the other treatments was more-or-less equal. The foliar pyrethroid application showed no sign of efficacy on either aphid species and consequently, also no effect on virus transmission. But any reduction in effects is also dependent on the time when immigration takes places.

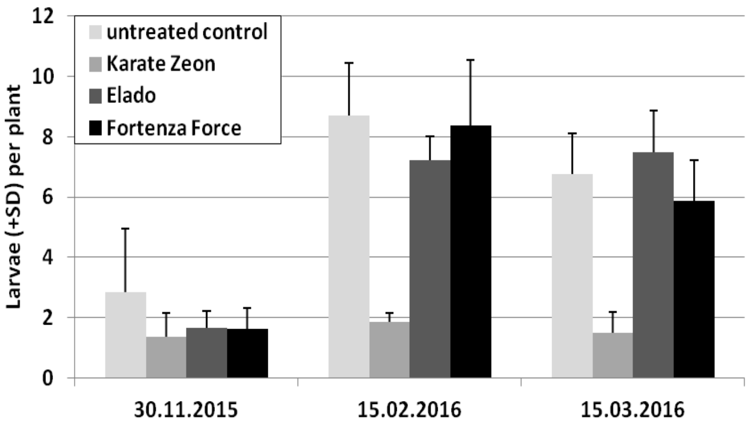

Figure 1. Influence of different treatments on the number of *P. chrysocephala* larvae per plant.

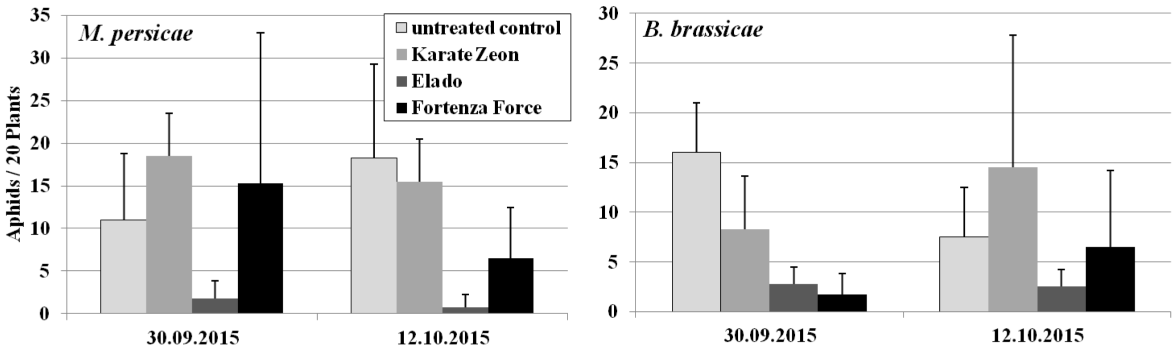

Figure 2. Influence of different treatments on the number of *M. persicae* (left) and *B. brassicae* (right) per 20 plants.

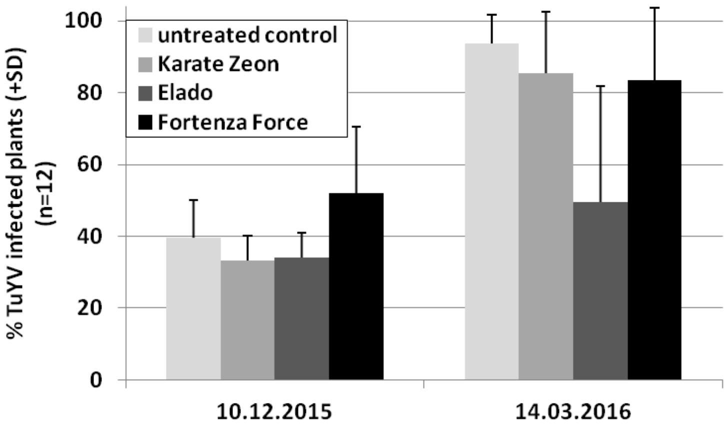

Figure 3. Impact of different insecticide treatments on the incidence of TuYV.

Acknowledgements

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Arabidopsis thaliana as a model plant for studying plant pathogen interactions during *Plasmodiophora brassicae* infection

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Abstract: *Plasmodiophora brassicae* is a biotrophic pathogenic protist infecting plants mainly from the Brassicaceae family. Its life cycle is tightly linked to soil and water environments. Resting spores of this pathogen can survive in the soil for many years and germinate once optimal conditions for infection occur. The first step of infection is mediated by haploid zoospores invading root hairs of the host plant. Inside the plant the pathogen develops multinucleate zoosporangia that produce plasmodia which are released into the soil and act as a source of secondary infection. Secondary zoospores invade the underground parts of a plant and develop into motile secondary plasmodia which are responsible for further disease spread and progression within the plant. Early stages of secondary infection are accompanied by massive changes in the host metabolism leading to establishment of a new, pathogen-oriented, physiological sink. As a consequence of these changes, serious hyperplasy followed by hypertrophy is observed. This leads to the development of galls on the underground parts of infected plants. During later stages of gall development giant cells are formed. These cells are the site of spore maturation. Once spores are mature, disintegration of the underground parts occurs and spores are released into the soil. Clearly, the completion of the entire life cycle by the pathogen is accompanied by complex reprogramming of the host. A detailed understanding of this reprogramming at the cellular and molecular levels can help to reduce the negative impact of *P. brassicae* on plant yield. Here we would like to discuss the usefulness of *Arabidopsis thaliana* as a model plant for studying complex host reprogramming by *P. brassicae*. *Arabidopsis thaliana* is a plant whose cellular and molecular regulation is well described. Due to the certain features of roots and hypocotyl *Arabidopsis* is a perfect model to localise and assign particular cellular changes to known molecular networks governing processes like cell proliferation, growth or differentiation. In our group we have developed a model describing how *P. brassicae* modulates certain cellular processes in host plants in order to usurp their nutrition and use it as a site for multiplication. The second advantage of *A. thaliana* is the possibility to use a wide range of molecular biology tools and resources which help to interpret each step of the plant-pathogen interaction. At the moment we are set up to study the infection from the very early to late stages of its progress. We recognise that certain significant differences between our model and crop plants such as oilseed rape do arise, therefore an opportunity to discuss these with oilseed rape experts at this IOBC meeting is of great importance to us.

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Quantifying the non-fungicidal effects of boscalid dimoxystrobin co-formulation in winter oilseed rape

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Abstract: The objective of good agronomic practice is to optimise the source availability of a crop relative to its sink capacity. Evidence suggests that many oilseed rape crops tend towards source limitation. The challenge therefore is to maximise the photosynthetic capacity in order for the crop to achieve its yield potential. Leaf area is a major determinant of yield in oilseed rape. Drastic declines in leaf area index usually occur from flowering onwards and can be exacerbated by disease epidemics, early senescence and pest damage. Decreases in canopy size and duration, and thus cumulative absorption of photosynthetically active radiation, subsequently reduce the amount of assimilate available for seed filling and have a negative impact on yield.

The active ingredient boscalid (BASF) belongs to the chemical group of carboxamides and is a succinate dehydrogenase inhibitor (SDHI) fungicide. Boscalid is marketed in co-formulation with dimoxystrobin (strobilurin, QoI) and has efficacy against the key diseases of oilseed rape (*Brassica napus)*: sclerotinia stem rot, light leaf spot, phoma stem canker and alternaria spot. However, yield increases have been reported following application of the fungicide that exceed expectations from disease control alone. The relationship between disease symptoms and yield loss varies across sites and seasons but can be strengthened when accounted for via the effects on green area index, canopy duration and accumulated light interception.

Eight field experiments were conducted across three cropping seasons in the UK (2012-2014). The objective was to quantify the effects of fungicide on yield, mediated through means other than the control of visible disease. The boscalid-picoxystrobin co-formulation was applied at mid flowering (BBCH 65) and evaluated against boscalid, prothioconazole, boscalid-metconazole co-formulation and an untreated control. Disease incidence and severity was assessed and physiological measurements included photosynthetic rate, transpiration efficiency, water use efficiency measured at the canopy and leaf level, leaf retention, leaf greenness, leaf and pod green area indices (integrated through time to give ‘healthy area duration’ [HAD] values), intercepted light, canopy senescence, lodging and yield.

Sclerotinia stem rot and light leaf spot incidence ranged from 0-50% across the experiments. Cross-site analysis showed that yield was significantly higher in boscalid-picoxystrobin treatments by a mean of 0.23 t/ha after disease effects had been accounted for. This was achieved through increases in both seed size and seed number. Effects of boscalid-picoxystrobin treatment on the canopy were significant. HAD was increased as a result of increases in green canopy area (largely through better green leaf retention) and canopy duration (due to delayed senescence). A strong positive association was found between yield and HAD with each unit of HAD increasing yield by approximately 0.06 t/ha. Consistent trends were observed for increased leaf and pod greenness, indicating a higher chlorophyll content following fungicide application.

Significant beneficial effects of boscalid-picoxystrobin on water use efficiency (WUE) were found at the canopy and leaf level. Effects on WUE were largely driven by improvements in yield for a given amount of water uptake which could be a useful mechanism for yield improvement on drought prone land where water is limited during the critical seed fill period. The results suggest that, in addition to controlling visible disease symptoms, the boscalid-picoxystrobin co-formulation is able to exert positive physiological effects on oilseed rape. The yield increase exceeds that which can be explained solely through good disease control and the associated loss of healthy canopy area.

Key words: pictor, boscalid, dimoxystrobin, sclerotinia, physiological effect, plant health

The potential of entomopathogenic fungi in biological control of oilseed rape pests

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Abstract: Oilseed rape is an important arable crop in many parts of the world, including central and northern Europe. The crop is attacked by a wide range of insect pests, many of which are of considerable economic importance. Traditionally, such pests have been targets for the application of insecticides. However, nowadays, there is an increasing demand to reduce chemical inputs on arable crops, and an increasing awareness of the potential benefits to be gained from the adoption of non-chemical methods for pest control within sustainable crop management strategies.

Entomopathogenic fungi can play an important role in the regulation of arthropod pest populations of many crops. The fungal species *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria farinosa*, *I. fumosorosea* and *Lecanicillium* sp. have been reported to be effective against some oilseed rape pests. Of these, the first two species have been the most studied and show potential for biological control of *Meligethes aeneus*, *Psylliodes chrysocephala* and *Ceutorhynchus obstrictus* (syn. *assimilis*). In order to develop sustainable pest management methods for arable crops based on entomopathogenic fungi (EPF), their efficacy, persistence and compatibility with pesticides, predators and parasitoids should be investigated.

In this work the presence of entomopathogenic fungi in the soils from field of winter oilseed rape was studied. Two methods were used to find and identify the species and to evaluate the density of entomopathogenic fungi (colony forming units) in each soil sample: (i) baiting soil samples with larvae of waxmoth (*Galleria mellonella*) and (ii) use of selective medium. In total, four entomopathogenic fungal species were isolated from investigated soil samples: *B. bassiana*, *I. fumosorosea, M. anisopliae* and *Lecanicillium* sp. The identification with the use of conventional methods was confirmed using ITS1-5.8s-ITS2 sequencing. Additionally, the efficacy of several isolated fungal strains against *M. aeneus* and *Ceutorhynchus* ssp. were studied in laboratory conditions. The results indicate that *B. bassiana* and *M. anisopliae* strains were highly pathogenic to tested oilseed rape pests.

In this presentation, the advantages and difficulties related to the potential use of pathogenic fungi for control of insect oilseed rape pests will be discussed.

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Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana* – the tricky path to an efficient formulation

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Abstract: Pollen beetles are a main pest in oilseed rape throughout Europe. A laboratory screening of Swiss isolates of entomopathogenic fungi revealed high potential of *Beauveria bassiana* for biological pollen beetle control. To improve efficacy of fungus spore suspensions in the field, we explored formulations with vegetable oils or stone dusts that have previously been shown to reduce pollen beetle abundancy in the field. The combined application of fungus spores and vegetable oil showed increased pollen beetle mortality in laboratory experiments and indicated increased yield in a field trial. To reduce fungus spore inactivation by ultraviolet irradiance upon field application, natural compounds were tested as UV-protectant additives to spore formulations. Formulation of *B. bassiana* spores with natural UV protectants tested in laboratory bioassays increased the number of surviving spores up to a factor of two, relative to untreated spores.

Key words: *Beauveria bassiana*, entomopathogenic fungi, formulation, *Meligethes* spp., vegetable oil, synergistic interaction, UV irradiance, UV protectant

Introduction

Pollen beetles (*Meligethes* spp.) cause substantial yield loss in oilseed rape throughout Europe. Increasing insecticide resistance and lack of sustainable control options urge the development of novel treatment methods. In previous studies with the entomopathogenic fungus *Beauveria bassiana*, high pollen beetle mortality in laboratory experiments but no significant increase in yield in field trials was seen (Kuske *et al*., 2011).

To improve efficacy, we explored the synergistic interactions between *B. bassiana* spores and vegetable oils or stone dusts that have previously been shown to affect adult pollen beetles (Daniel, 2011; Dorn *et al*., 2014). The combined application of fungus spores and vegetable oil achieved increased and faster pollen beetle mortality in laboratory experiments (Kaiser *et al*., 2016). Results from a field experiment indicated a reduction in pollen beetle numbers, higher numbers of pods and increased yield.

However, field samples showed a decrease in *B. bassiana* spore viability of 90% after three days, which is probably due to the susceptibility of spores to ultraviolet irradiance. Consequently, we examined natural compounds to protect the fungus' spores from ultraviolet irradiance when applied in the field. Promising substances were tested as UV-protectant additives to spore formulations.

Material and methods

Fungal strain

Beauveria bassiana strain ART2587 was isolated in 2004 from a mycosed pollen beetle collected in Zurich, Switzerland (Pilz, 2005). The isolate was frozen and integrated into the Agroscope strain collection. Before the start of the experiments, the fungal strain underwent a host passage through pollen beetles and was re-isolated from single conidia colonies.

Field trial

Submersed spores of *B. bassiana* strain ART2587 were used as single treatment with a concentration of 10^{14}/ha or in combination with 2% Telmion (commercial wetting agent containing 85% oilseed rape oil). Formulations were tested with five repetitions of $300m^2$ size each, arranged in a completely randomized block design. Persistence of viable fungus spores in field applications was assessed by plating processed plant material on agar plates and counting grown colony forming units.

UV irradiance of Beauveria bassiana spores

Effect of UV protectants on *B. bassiana* spore survival upon UV irradiance was tested in a climate chamber to ensure stable temperature and humidity. Fungus spore formulations were applied to agar plates of SDA containing 1% yeast extract or on oilseed rape leaf discs. Agar plates and leaf discs were exposed to artificial UV light of 40 kJ/m^2 and 54 kJ/m^2 UV-B, respectively (strip lights, SolarRaptor T5UVB). Applied irradiation intensities correspond to UV-B irradiances measured in Switzerland lowland mid of April during 2 and 2.5 days. Control replicates were placed in the same climate chamber protected from UV irradiance by aluminum separation. As outcome, grown colony forming units were counted. Treated leaf discs were homogenized beforehand and suspension spread on agar plates.

Results

The combined application of *B. bassiana* spores and vegetable oil in a field trial caused a decrease of 47% in adult *Meligethes* spp. abundance three days after application. Furthermore, treated plants showed 1.4 times more pods on main shoots and an increase in yield of 4.2 dt/ha was seen.

Fungus spores isolated from collected plant material showed a decrease in persistence of 90% and 100% three and seven days after field application, respectively.

Formulation of *B. bassiana* spores with natural UV protectants tested in laboratory assays increased the number of surviving spores up to a factor of two, relative to untreated spores. Screenings of further potential UV protectants in various concentrations are currently ongoing in laboratory scale bioassays and one promising formulation was included in a field experiment in the 2016 growing season.

Acknowledgements

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Reducing pesticides in oilseed rape production – A multisite long-term field experiment in Luxembourg

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Key words: Herbicides, cropping system, weed communities

Introduction

Winter oilseed rape (*Brassica napus* L.) is an important crop in Luxembourg grown on 5000 ha on average per year. The main advantages of cultivating oilseed rape are: (1) reliable yields of approx. 42 dt/ha (2) importance as a break crop in cereal-based rotations (3) importance as a food resource for insects and (4) continuous soil cover throughout the year to prevent soil erosion. On the negative side, the production of winter oilseed rape (WOSR) is characterized by intense usage of fertilizers and pesticides.

Based on regular ground and surface water-analysis, a high level of contamination by transformation products of WOSR specific herbicide compounds were found in Luxembourg. Therefore, scientific, administrational and advisory institutions of the agriculture business defined a multi-site multi annual collaborative project. The objectives are: (1) to identify suitable cropping techniques to reduce the amount of highly mobile herbicides and transformation products used in WOSR and (2) to explore the potential substitutes as oil producing crops such as false-flax (*Camilina sativa* L.) and flax (*Linum usitatissimum* L.*).*

Material and methods

Three experimental sites in Luxembourg were chosen based on their soil properties: Hobscheid, Reisdorf and Flatzbour. On each of the sites, eight different cropping techniques and potential substitutes for WOSR were cultivated in a randomised block design with four replicates each.

1. WOSR with applications of Metazachlore
2. WOSR with applications of a suite of alternative herbicides to Metazachlore
3. WOSR with under sowing (*Colza associé*)
4. WOSR in extended row space (75 cm) with herbicide applications only within rows
5. False-flax (*Camilina sativa*)
6. Flax (*Linum usitatissimum*)
7. WOSR with mechanical weeding
8. WOSR in biological cultivation without chemical control measures

Continuous field monitoring including: (1) growth stages and plant development (2) yellow trapping of pest insects (3) soil sampling and analysis of herbicides residues (4) weed community monitoring and (5) leaf area measurements were done.

Results and discussion

Preliminarily results concerning the number of plants per square meter and the root collar diameter indicate significant differences between the three experimental sites. The number of plants per square meter in Hobscheid and Reisdorf were in a comparable range for all experimental varieties, whereas the plant density at Flatzbour was significantly lower. This might be attributed to local micrometeorological conditions and different intensities of root maggots of cabbage root fly, *Delia brassicae* L., in autumn. In contrast, the root collar diameter at Hobscheid and Flatzbour were in comparable ranges (7.6 cm) but clearly lower than at Reisdorf (11.3 cm).

The monitoring of the weed communities was done at all three sites in autumn and spring (after weed control measures). Results of four out of the eight experimental treatments (Metazachlore, herbicide alternatives, extended row space and biological cultivation) are presented here. Overall up to 25 different weed species were identified. Experimental sites showed significant differences concerning the species distribution as well as in the number of species and total number of weeds per square meter. Reisdorf showed the lowest total number of weeds followed by Hobscheid and Flatzbour. Chickweed (*Stellaria media*) and Knotweed (*Polygonum* spp.) were mainly found in autumn. A tendency towards brassicaceous weed species was not found except for Shepherd’s purse (*Capsella bursa-pastoris*) that was mainly found in springtime at Flatzbour. Species that were mainly observed in autumn disappeared due to the chemical and mechanical control measures and were replaced by other weed species in the following spring e.g. Field pansy (*Viola arvensis)*, Common poppy (*Papaver rhoeas*) and Poisson hemlock (*Conium maculatum*). Both herbicides Metazachlore and the alternative herbicides did not perform well at Hobscheid due to the sandy soil characteristics. Results are still preliminary and will be validated in the next four years of the project.

Acknowledgements

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Entomology Session 1 –
Insecticides: need, efficacy and side effects

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Field trials to assess the short-term and long-term effects of several insecticides used to control the pollen beetle on parasitic hymenoptera in oilseed rape

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Abstract: Large scale field trials were performed in spring 2013, 2014 and 2015 to assess the effects of Pymetrozine (2013-2015), Tau-fluvalinate (2013-2015), Thiacloprid (2013-2015), Chlorpyriphos-ethyl (2013-2015), Phosmet (2013-2014) and Indoxacarb (2015), used to control the pollen beetle *Meligethes aeneus* (F.) (Col.; Nitidulidae), on pollen beetle populations and their parasitic wasps in winter oilseed rape. The insecticides were applied at the recommended rate for commercial use soon 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 weekly using beating methods and sweep netting from day 1 to 50 days following product application. The direct effects of the products were assessed on adult pollen beetles (target pest) and adults of parasitic hymenoptera. The 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 each product scenario for the next season.

According to the results obtained, the insecticides were split in two categories. The first were products such as Pymetrozine, Tau-fluvalinate, Phosmet and Indoxacarb that had no long term effects on pollen beetle parasitism; these products could therefore be used to control the pollen beetle within an IPM strategy, even if short term effects were sometimes observed. The second category included Thiacloprid and Chlorpyriphos-ethyl. These products were found to significantly impact pollen beetle parasitism at the end of the growing season and could therefore be considered as a threat to parasitic wasps, with parasitism rates reduced by 60% to 100% compared with controls, depending on the year and the product.

Despite different agricultural conditions, the date of application of products, pest pressure and parasitism rates, the results were consistent from year to year for products that were assessed several times, as well as for active ingredients tested with different formulations. A ‘positive’ list of products that are both effective in control of the pollen beetle but which did not negatively affect parasitism rates in the long term was edited on the basis of these results.

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Pyrethroid resistance of insect pests in oilseed rape in Germany since 2005

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Abstract: Pyrethroid resistant pollen beetles (*Meligethes aeneus*) are now widely distributed in Germany since the first resistant beetles were detected about 15 years ago. Over half (53%) of sensitive populations in biotests in 2005 had declined to 0% by 2011. Very clear resistance increased from 33% in 2005 to 100% in 2015. Monitoring of the other insect pests of oilseed rape in Germany showed no resistance yet in pest species including *Ceutorhynchus napi* and *C. pallidactylus*, *Dasineura* spp. and *Phyllotreta* spp., but resistance to pyrethroids has recently developed in *C. obstrictus* and *Psylliodes chrysocephala*. Resistance of both species has spread over many parts of Germany but resistance factors in laboratory biotests are far below resistance values known for *M. aeneus.*

Key words: pyrethroid resistance, pollen beetle, cabbage stem flea beetle, cabbage pod weevil

Introduction

Reasons for the fast development and spread of insecticide resistance include limitations in the availability of insecticides with different modes of action for use in oilseed rape, high pests infestation pressure in some years and regions, several target insect pests (and growth stages) which occur over a long period during the crop development, and too many prophylactic sprays which ignore damage thresholds. In Germany, for many years the only authorized active ingredients in floliar sprays for oilseed rape were pyrethroids. In addition for many years only pyrethroids were able to provide sufficient control for some of the pests occurring during the growing season and some were safe for bees and could be applied during the flowering period. However, pyrethroid resistance has developed for several insect pests of oilseed rape in Germany (Heimbach & Müller, 2013) and pollen beetle resistance spread over Europe (Slater *et al*., 2011). The resistance mechanism for pollen beetles (*Meligethes aeneus*) has been identified as target site and enhanced metabolisation (Zimmer *et al*., 2014) and for the cabbage stem flea beetle (*Psylliodes chrysocephala*) kdr plus for UK populations an additional unknown mechanism (Höljland *et al*., 2015); for the cabbage pod weevil (*C. obstrictus*) and *C. picitarsis* kdr has been detected (Elias, oral communication). Monitoring of resistance is therefore an important tool for the early detection of arising resistance problems and for developing anti-resistance management strategies.

Material and methods

Since 2005, pest insects have been collected in oilseed rape from different regions of Germany and tested in biotests (slightly changed adult vial test (AVT), IRAC 11) for pyrethroid sensitivity. In all tests, l-cyhalothrin was used as active substance representing class II pyrethroids; in many tests other active substances like etofenprox or tau-fluvalinate were also used. Up to 4 replicates for several rates of the active substances, was applied to glass vials and about 10 adults per vial were released. In all regions of Germany participating in biotests, the AVT was carried out using testkits with coated glasses supplied by the JKI that had been kept under cool and dark conditions before the test and using a standard protocol. After 5 hours of exposure at 20 °C and light conditions, the number of unaffected and effected beetles was assessed. As validity criteria, not more than 20% control mortality was used. LD_{50} values were calculated whenever possible using the Polo Plus program.

Results and discussion

The results of the monitoring (Figure 1) show a decline of susceptible pollen beetle populations since 2005. The level of resistance increased from year to year. In 2015, more than 90% of pollen beetle populations were highly resistant in Germany. No pyrethroid susceptible populations were found since 2010. This is probably the result of pyrethroids still dominating the insecticide market which at that time lacked products with other active ingredients with alternative mode of action.

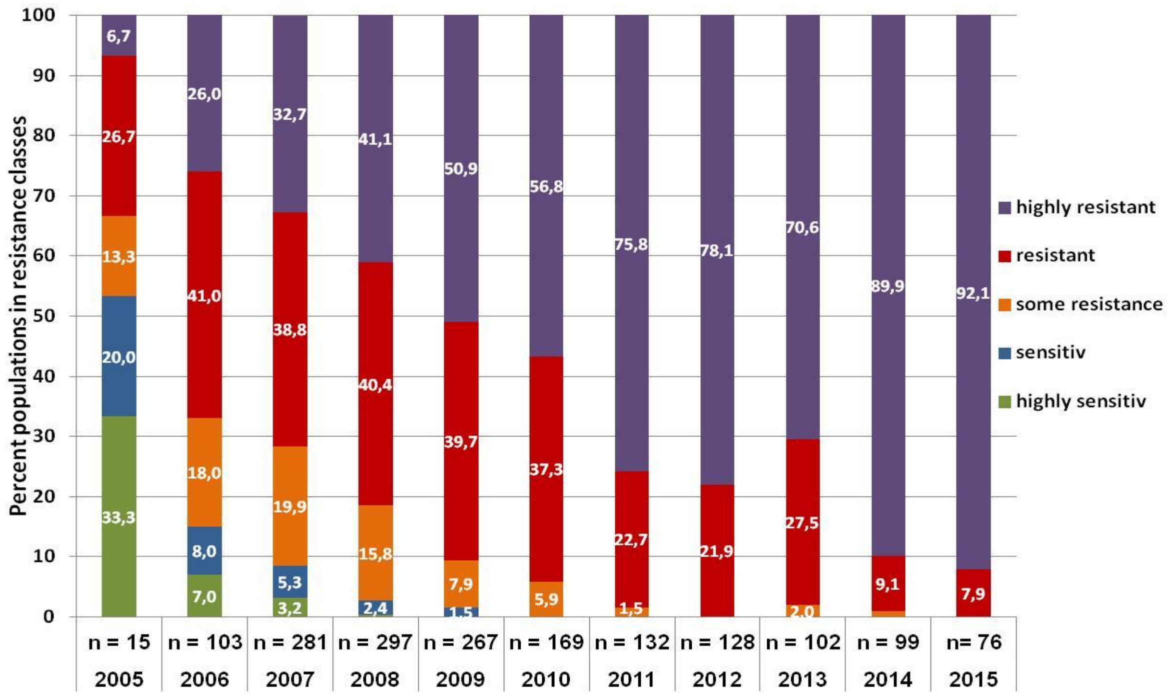

Figure 1. % Pollen beetle populations classified in pyrethroid resistance classes (highly resistant, resistant, some resistance, sensitive or highly sensitive) in Germany 2005-2015 from Adult Vial Tests (lambda-cyhalothrin, 5 h assessment in AVT, control mortality < 20%)

The data of the AVT showed no clear cross-resistance between class I pyrethroids (bifenthrin, etofenprox, tau-fluvalinate) and l-cyhalothrin (used as representative for class II pyrethroids). Nevertheless, from 2008 to 2015 a decreasing mortality (Figure 2) in the AVT was observed also for the class I pyrethroid etofenprox, which was used on a large scale in Germany and less clear for tau-fluvalinate and bifenthrin (data not shown), both being used on a much smaller acreage.

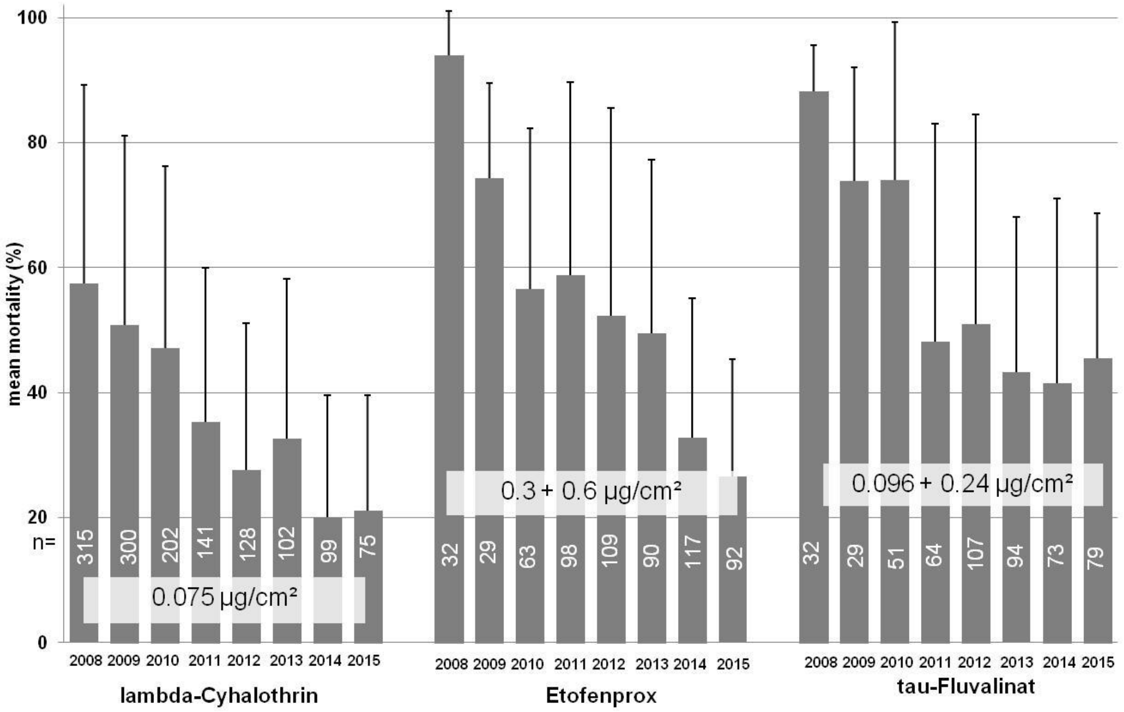

Figure 2. Mean % mortality of pollen beetle populations in Germany 2008-2015 at different rates of pyrethroids (5 h assessment in Adult Vial Tests, control mortality < 20% using lambda-cyhalothrin 100%, etofenprox 50 + 100%, and tau-fluvalinat 20 + 50% of the field rate registered in Germany).

The mortality of more than 100 populations of *Ceutorhynchus napi* and *C. pallidactylus* at a dose of 0.015 µg/cm² lambda-cyhalothrin (20% of the field rate registered in Germany) was, in most cases, 100% with limited variability between populations; populations of *C. pallidactylus* generally showed less sensitivity than *C. napi* (data not shown). Also for some populations of *D. brassicae* and *Phyllotreta* spp., no clear signs of resistance were detected with 100% effect using higher rates than 4% of the German field rate (data not shown).

Some populations of *C. obstrictus* especially those in the north of Germany, differed from the majority of test populations from about 2009 onwards. Control failures of pyrethroid applications in the field were reported. In 2015 pyrethroid resistance has spread over most parts of Germany (Figure 3). To calculate a resistance factor, the mean of the LD_{50} of the 10 most resistant populations was divided by the mean of the 10 most sensitive populations. Thus a more realistic factor can be achieved if no standard sensitive laboratory population is available as for most pest insects of oilseed rape. Building a mean of several populations reduces the influence of the quite high variability of each single LD value. The resistance factor in the AVT was 63.5 of together 83 populations for which a LD value calculation was possible. Kdr has been shown to be present in resistant *C. obstrictus* populations (oral communication by J. Elias, Syngenta).

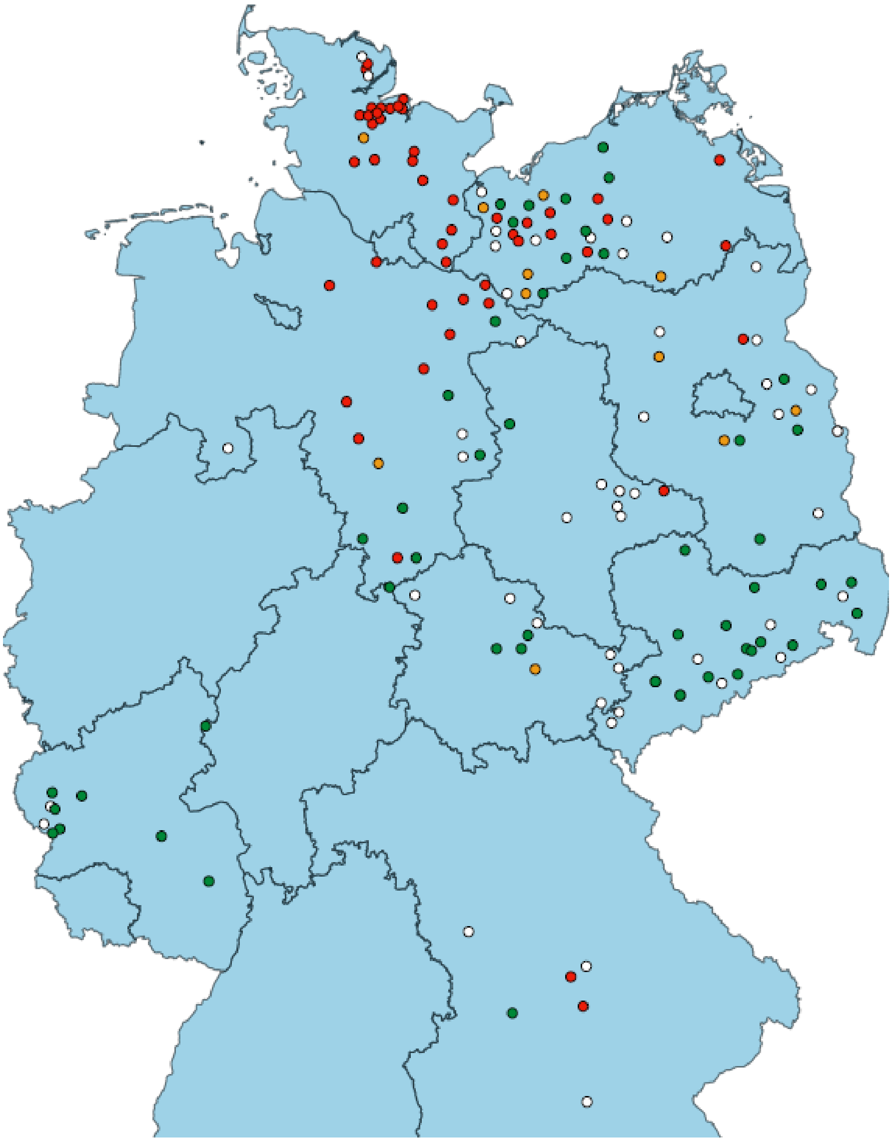

Figure 3. Distribution of sensitive (green/white points) and resistant (orange/red points) populations of *Ceutorhynchus obstrictus* in Germany 2010-2015 (5 h assessment in Adult Vial Tests, control mortality < 20%).

For *Psylliodes chrysocephala* the first case of resistance occurred in 2008, again in the north of Germany with reduced field performance of pyrethroids reported and a resistance factor of 20.6 comparing mean LD_{50} value of the 10 most sensitive to the 10 least sensitive populations of together 65 populations for which a LD calculation was possible. Resistance nowadays is present in most parts of Germany (Figure 4) either shown by AVT or kdr analysis (carried out by R. Nauen, BayerCropScience or J. Elias, Syngenta). The resistance factor is expected to be distinctly higher in UK populations with another resistance mechanism present (Højland *et al*., 2015).

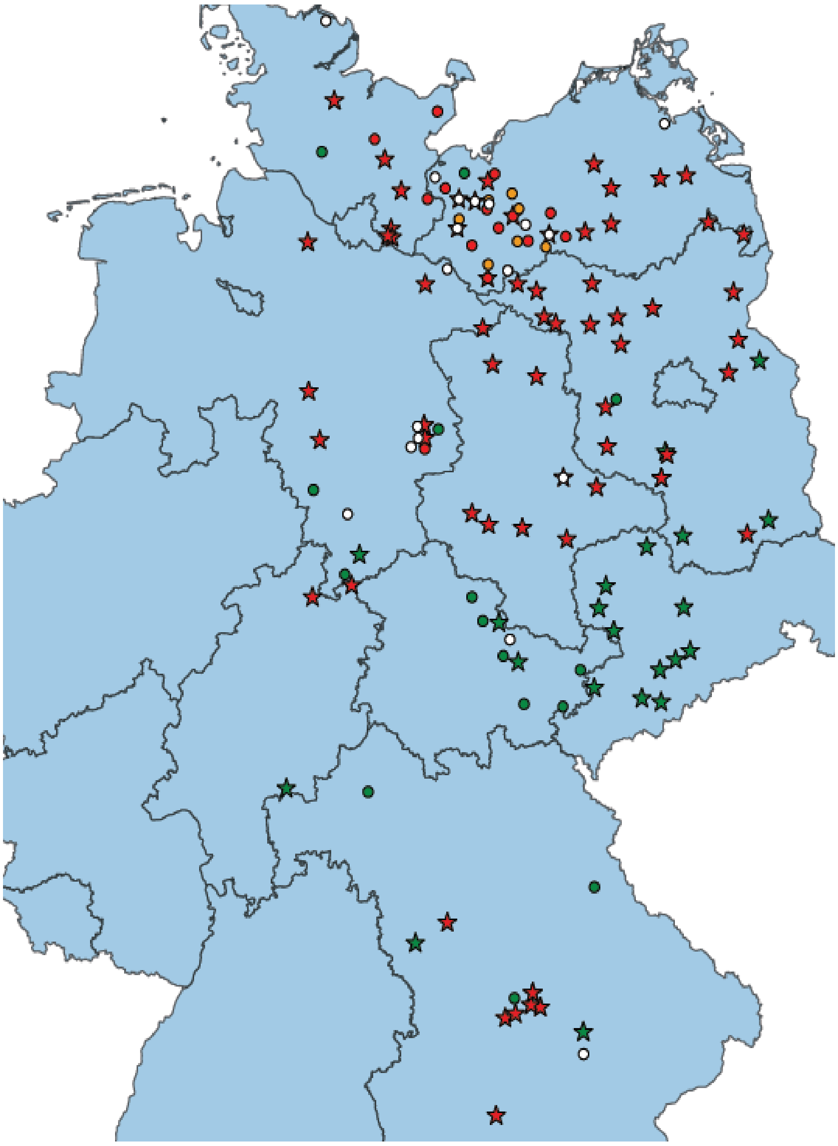

Figure 4. Distribution of sensitive (green and white points in Adult Vial Tests, green star in kdr analysis) and resistant (orange/white points in AVT, red stars in kdr analysis) populations of *Psylliodes chrysocephala* in Germany 2010-2015 (5 h assessment in AVT, control mortality < 20%), kdr analysis by R. Nauen and E. Elias.

The monitoring activities have shown an increasing severity of pyrethroid resistance in Germany, with a spread of resistance from the northern areas and more pest species being affected. There is an urgent need for new insecticidal modes of action and more targeted use of insecticides by farmers to reduce the selection pressure for resistance. New control methods including non-chemical options need to be developed to allow sustainable oilseed rape production.

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Pyrethroid resistance in cabbage stem flea beetle (*Psylliodes chrysocephala*) and rape winter stem weevil (*Ceutorhynchus picitarsis*) populations in France

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Abstract: The cabbage stem flea beetle and rape winter stem weevil are two major pests of winter oilseed rape (WOSR) in France. Since 2009, certain areas of France have suffered from increased pest infestations despite repeated treatments to control them. The phenomenon has increased year after year. Terres Inovia and its partners, through the AFPP (French Association for Plant Protection) “Insect resistance” group, have confirmed that several populations of these two pests are resistant to pyrethroids and that multiple mechanisms are involved. A mutation in the sodium channel gene known to be linked to the knock-down resistance (kdr) phenotype was discovered in the cabbage stem flea beetle. The mutation is widely spread in French territories where resistance is found. A second mutation known as Super-knock down resistance (skdr) was also detected in the pest. This mutation is found in the East of France, mainly in the Yonne region, but its distribution seems to be bigger. For rape winter stem weevil, the kdr mutation was found but not skdr. Furthermore, we showed with inhibition tests that resistance by detoxification is combined with these mutations to confer a high level of resistance to lambda cyalothrin in these two pest species. A complex combination of kdr, skdr and metabolic based resistance seem to confer different levels of resistance in the field.

Over reliance on chemical insecticides will only serve to increase the spread of resistance in the cabbage stem flea beetle and rape winter stem weevil. Agronomical practices are the best way to reduce the impact.

Key words: *Psylliodes chrysocephala*, *Ceutorhynchus picitarsis*, resistance, pyrethroids, agronomical practices

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Correlations between susceptibilities to lambda-cyhalothrin and chlorpyrifos-ethyl with respect to thiacloprid in Czech populations of *Meligethes aeneus*

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Abstract: Populations of Czech *Meligethes* were simultaneously tested for susceptibility to lambda-cyhalothrin and chlorpyrifos-ethyl and also to lambda-cyhalothrin and thiacloprid in 2014. In 2015, *Meligethes* populations were again tested for susceptibility to lambda-cyhalothrin and chlorpyrifos-ethyl and to lambda-cyhalothrin and thiacloprid simultaneously, this time using populations of mainly Czech but also Slovak origin. IRAC adult vial tests were used. IRAC no. 011 v. 3 for lambda-cyhalothrin, IRAC no. 025 for chlorpyrifos-ethyl and IRAC no. 021 for thiacloprid. For each of the tested populations the LC_{50} and LC_{90} values for the three insecticides were determined. Correlation analyses were made with transformed (log_{10}) LC values. Significantly negative correlations were recorded for the LC_{90} and LC_{95} values estimated for the correlation between lambda-cyhalothrin and chlorpyrifos-ethyl. Pyrethroid resistance in pollen beetle populations should indicate their slightly higher susceptibility to chlorpyrifos-ethyl. Contrary to expectations, significant positive correlations were recorded for the LC_{50}, LC_{90} and LC_{95} values for the correlation between lambda-cyhalothrin and thiacloprid in both years. Pyrethroid resistance in pollen beetle populations should indicate their lower susceptibility to thiacloprid.

Key words: pollen beetles; IRAC adult vial tests; pyrethroid resistance; lambda-cyhalothrin, chlorpyrifos-ethyl, thiacloprid

Introduction

Progressive spread of pollen beetle (*Meligethes aeneus* F., Coleoptera: Nitidulidae) populations resistant to pyrethroids through the various countries and regions of Europe has been described and documented in many papers (e.g.: Derron *et al*., 2004; Ballanger *et al*., 2007; Djurberg & Gustafsson, 2007; Wegorek *et al.*, 2006; Wegorek *et al.*, 2009; Philippou *et al*., 2011, Eickermann *et al*., 2008; Thieme *at al*., 2006; Heimbach *et al*., 2007; Thieme *at al*., 2008, Heimbach & Müller, 2013). Seidenglanz *et al*. (2015a,b) documented the spread of resistant populations in the Czech Republic (CZ) and also partly in Slovakia (SK) from 2009.

In contrast to the situation with pyrethroids, European pollen beetle populations seem to be fully susceptible to the organophosphate chlorpyrifos-ethyl although the active ingredient has also been used for the control of insect pests in oilseed rape for many years. Whilst information has been published regarding the lower susceptibility (or resistance) of pollen beetles to some organophosphate insecticides it originates from Poland (Lakocy, 1967; Wegorek *et al*., 2009), and has not been documented elsewhere in Europe (http://www.irac-online.org).

The resistance of European *Meligethes* populations to thiacloprid has not been reported yet. Between 2009 and 2012, Zimmer & Nauen (2011), and Zimmer *et al.* (2014) observed there was no shift towards lower susceptibility of pollen beetles to thiacloprid. They tested more than 630 populations of pollen beetle from 13 different countries using the adult vial test. Thieme *et al.* (2010) also observed no resistance in Bavarian populations from Germany. Thieme considers contrary information reporting the loss of effectiveness of thiacloprid in controlling high infestations of pollen beetles, under field conditions, in Germany in 2007 to be a rumour; if true this implies a decline in effectiveness started relatively soon after the registration of thiacloprid.

Although, most of the CZ and SK populations have generally shown high levels of resistance to lambda-cyhalothrin in recent years, the LC_{50} - LC_{95} values stated for the active ingredient have often been significantly different in individual populations (Seidenglanz *et al*., 2015a; b). Similarly, LC_{50} - LC_{95} values estimated for chlorpyrifos-ethyl and thiacloprid in the same populations have not been uniform either (Seidenglanz *et al*., 2015c). Hence, some questions emerge: Are the populations with higher levels of resistance to esteric pyrethroid lambda-cyhalothrin less or more susceptible to organophosphate chlorpyrifos-ethyl and neonicotinoid thiacloprid respectively? And how could this knowledge be used to inform best use of chlorpyrifos-ethyl and thiacloprid in future Insecticide Resistance Management programmes for oilseed rape?

Material and methods

Sixty-five Czech *Meligethes* populations were simultaneously tested to lambda-cyhalothrin and chlorpyrifos-ethyl and thiacloprid in 2014 (Figure 1). In 2015, sixty *Meligethes* populations were tested against lambda-cyhalothrin and chlorpyrifos-ethyl and fifty-eight populations tested against lambda-cyhalothrin and thiacloprid. Some of the populations tested in 2015 were sampled in Slovakia (Figures 2a, b).

Adult vial tests, as recommended by the Insecticide Resistance Action Committee (IRAC) were used for resistance tests. To test the susceptibility of pollen beetles to lambda-cyhalothrin, test no. 011 v. 3 was used. In contrast to the methodology an additional concentration (the highest) was also used. The concentrations tested were: 0 g a. i./ha = untreated control; 0.3 g a. i./ha; 1.5 g a. i./ha; 7.5 g a. i./ha (recommended field rate in Europe); 37.5 g a. i./ha and 112.5 g a. i./ha. For chlorpyrifos-ethyl test no. 025 was used. In contrast to the methodology substantially more concentrations were used in tests. The concentrations tested were: 0 g a. i./ha = untreated control; 0.3 g a. i./ha; 0.9 g a. i./ha; 2.9 g a. i./ha; 30.0 g a. i./ha; 96.0 g a. i./ha and 307.2 g a. i./ha (approx. field rate in CZ and SK). For thiacloprid (commercial formulation BISCAYA 240 OD was used according to the methodology) test no. 021 was used. Five concentrations (in agreement with the methodology) were tested: 0 g a. i./ha = untreated control; 2.9 g a. i./ha; 14.4 g a. i./ha; 72 g a. i./ha (recommended field rate in Europe) and 144 g a. i./ha. All the three laboratory methods are described in detail on http://www.irac-online.org/teams/methods/.

Figure 1. Distribution of 65 *Meligethes* populations from Czech area tested with both lambda-cyhalothrin and chlorpyrifos-ethyl and compared against lambda-cyhalothrin and thiacloprid simultaneously in 2014. Sixty-five pairs were therefore used for correlation analysis.

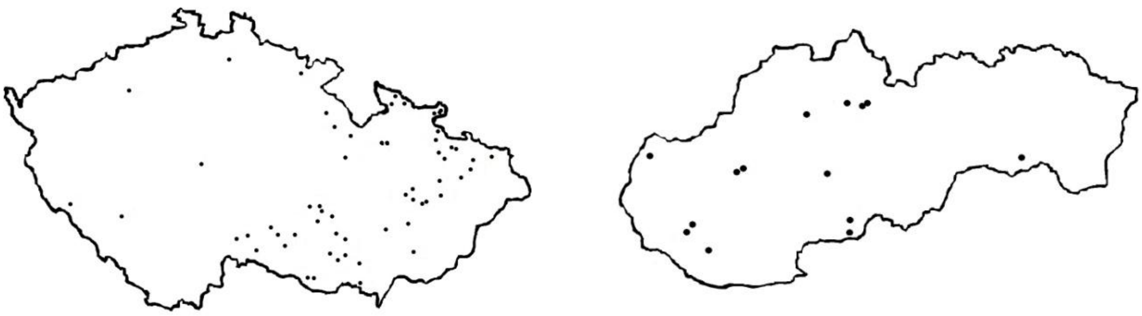

Figure 2a and b. The distributions of Czech (left hand map) and Slovak (right hand map) populations of *Meligethes* used in resistance testing in 2015. Sixty populations were tested with lambda-cyhalothrin and chlorpyrifos-ethyl and 58 with both lambda-cyhalothrin and thiacloprid simultaneously. Fifty-eight pairs were therefore used for correlation analysis.

For each population the values of LC_{50}, LC_{90} (and in 2015 also LC_{95}) were estimated for each of the three active ingredients in both years (2014 and 2015). Probit regression was used (Polo Plus v.2; LeOra Software, Berkeley, CA) for the calculations. The result was sixty-five pairs of simultaneously related LC values for correlation analysis in 2014 and sixty (or fifty-eight) in 2015. Correlation analyses were made with $\log_{10}$ transformed LC values. The reason for log transformation of LC values was to achieve a normal distribution of the data in the individual collections of LC values. Spearman´s correlation coefficients were determined ($p < 0.05$) for the related pairs of LC values (lambda-cyhalothrin $LC_{50,90,95}$ × chlorpyrifos-ethyl $LC_{50,90,95}$ and lambda-cyhalothrin $LC_{50,90,95}$ × thiacloprid $LC_{50,90,95}$). Calculations were made using Statistica software v.10 (STATSOFT, Inc. 1984-2013).

Results

The results of the correlation analysis for lambda-cyhalothrin and chlorpyrifos-ethyl are listed in Table 1. Correlation coefficients were negative in all cases and in three cases the negative correlation proved to be significant ($p < 0.05$): between LC_{90} and LC_{95} values in both years. However, the numerical values of the correlation coefficients (**r**) are relatively low even in the cases where the negative correlation proved to be significant. They vary from -0.37 to -0.43. This indicates a rather weak (or intermediate) correlation. In addition, the negative correlations for the LC_{50} values showed a very low intensity (**r** ranged from -0.04 to -0.12) and were insignificant ($p > 0.05$).

Table 1. Results of a correlation analysis between LC values estimated for the two active ingredients when compared simultaneously: lambda-cyhalothrin vs. chlorpyrifos-ethyl. In 2014 only Czech pollen beetle populations were tested. Both Czech and Slovak populations were included in tests in 2015.

Year	no. of analysed pairs of LC values	correlation analysis between values of:	correlation coefficient **r** [2]	probability value (**p**)
2014	65	[1]Log LC_{50}	-0.12	0.364
		[1]Log LC_{90}	**-0.41**	**0.001**
2015	60	[1]Log LC_{50}	-0.04	0.780
		[1]Log LC_{90}	**-0.37**	**0.003**
		[1]Log LC_{95}	**-0.43**	**0.001**

[1]Log transformation of the LC values estimated for the both active ingredients were made before the analysis
[2]bold values indicate the cases where the significant negative correlation ($p < 0.05$) was recorded between the analysed LC values

The results of the correlation analysis for lambda-cyhalothrin and thiacloprid are listed in Table 2. Correlation coefficient values were all positive and in four cases the positive correlation was significant ($p < 0.05$): between LC_{90} and LC_{95} values in both years and between LC_{50} values in 2014. The numerical values of the correlation coefficients (**r**) are relatively low, even where positively significant, with one exception where the value of (**r**) exceeded 0.5. They vary from -0.37 to -0.58. This again indicates rather weak (or intermediate) correlation intensity.

Table 2. Results of a correlation analysis between LC values estimated for the two active ingredients when compared simultaneously: lambda-cyhalothrin vs. thiacloprid. In 2014 only Czech pollen beetle populations were tested. Both Czech and Slovak populations were included in tests in 2015.

Year	no. of analysed pairs of LC values	correlation analysis between values of:	correlation coefficient **r** [2]	probability value (**p**)
2014	65	[1]Log LC_{50}	**0.44**	**0,000**
		[1]Log LC_{90}	**0.58**	**0,000**
2015	58	[1]Log LC_{50}	0.18	0.184
		[1]Log LC_{90}	**0.37**	**0.005**
		[1]Log LC_{95}	**0.39**	**0.003**

[1]Log transformation of the LC values estimated for the both active ingredients was made before the analysis
[2]bold values indicate the cases where the significant negative correlation ($p < 0.05$) was recorded between the analysed LC values

Discussion

It is immediately obvious from the results that lower susceptibility to lambda-cyhalothrin does not mean a predisposition to lower susceptibility to chlorpyrifos-ethyl in *Meligethes* populations. Furthermore, the results presented here even indicate higher susceptibility to chlorpyrifos-ethyl in the populations with higher levels of resistance to lambda-cyhalothrin.

Some other studies also demonstrate that lower susceptibility (or resistance) of pollen beetles to esteric pyrethroids does not increase the risk of lower susceptibility of the pest to chlorpyrifos-ethyl simultaneously (Philippou *et al.*, 2011; Slater *et al.*, 2011; Zimmer & Nauen, 2011; also on: http://www.irac-online.org). Wegorek & Zamoyska (2008) described and documented a strong negative cross resistance between pyrethroids and chlorpyrifos-ethyl in pollen beetle populations in Poland (also in Wegorek *et al*., 2009). The higher susceptibility to chlorpyrifos-ethyl in the pyrethroid resistant populations probably relates to the prevailing mechanism of resistance detected in populations from central Europe (Philippou *et al*., 2011). That is *metabolic resistance* which is based mainly on enhanced oxidative metabolism in less susceptible individuals (Obrepalska-Steplowska *et al*., 2006; Slater & Nauen, 2007). Oxidative enzymes (cytochrome P_{450} monooxygenases) play the most important role in detoxification of pyrethroids in insect bodies (Moores *et al*., 2009; Philippou *et al*., 2011). However, whereas with pyrethroids the oxidation results in detoxification of the active ingredients, oxidative desulfuration of chlorpyrifos-ethyl leads to the creation of a much more toxic metabolite. The results presented in this paper therefore support, in part, the conclusions made by the Polish authors (Wegorek & Zamoyska, 2008; Wegorek *et al*., 2009). However, contrary to their findings the results presented here indicate a rather weak intensity of the negative cross resistance between pyrethroids (lambda-cyhalothrin) and organophosphates (chlorpyrifos-ethyl).

Research demonstrating that higher levels of resistance against esteric pyrethroids can result in higher susceptibility to some organophosphates is especially important at this time when pollen beetle populations in Europe are becoming less susceptible to neonicotinoids. Wegorek & Zamoyska (2008) and Wegorek *et al*. (2009) recorded a wide occurrence of populations resistant to acetamiprid in Poland, Seidenglanz *et al*. (2015c) described significant shifts in pollen beetle susceptibility to thiacloprid among CZ populations during 2011-2015. Lower susceptibility to neonicotinoids could be explained in part by studies reporting that oxidative enzymes (cytochrome P_{450} monooxygenases) can play a similar role in resistance to neonicotinoids as in the case of pyrethroids (Jones *et al*., 2011). The mutual relationships between the susceptibility of pollen beetles to pyrethroids and neonicotinoids (thiacloprid, acetamiprid) could be substantially different to the relationship between pyrethroids and organophosphates. The results of this paper seem to support such conclusions and present the possibility that pyrethroid resistance in pollen beetle populations would result in lower susceptibility to thiacloprid. This is of great concern as neonicotinoides are perceived as the only real alternative for pyrethroids in Insecticide Resistance Management programmes in oilseed rape crops. However, the results should be interpreted very carefully because they contrast strongly with the study of Zimmer & Nauen (2011), who observed no trends of cross-resistance between lambda-cyhalothrin and thiacloprid. In their study even those populations classified as highly resistant to pyrethroids did not show any lower susceptibility to thiacloprid, suggesting a complete lack of cross-resistance.

The results of this paper indicate that chlorpyrifos-ethyl could be a suitable insecticide for use in Insecticide Resistance Management programmes in oilseed rape crops. Further work is necessary to determine whether cross-resistance between pyrethroids (lambda-cyhalothrin) and thiacloprid exists in pollen beetle populations.

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Effects of thiacloprid on population dynamics of pollen beetle in field studies 2013-2015

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Abstract: Pollen beetle (*Meligethes aeneus* F.) is one of the major insect pests on oilseed rape. The presence of high numbers of beetles during the green bud stage of the crop can result in substantial yield losses. Widespread resistance to pyrethroids throughout Europe has complicated its control. To achieve a sustainable effect on pest populations, insecticides are required not just for the effective direct control of overwintered pollen beetles but to simultaneously reduce the reproduction of the pest. The aim of this study was to determine the effect of the neonicotinoid Biscaya (a.i. thiacloprid) applied at the bud stage (BBCH 53-55) on the population dynamics of pollen beetle.

In 2013 -2015, field trials were conducted on crops of winter oilseed rape in the region of Braunschweig (northern Germany). Insecticide treated and untreated plots of approximately 1000 m^2 were established in a randomized block design with four replications. Overwintered pollen beetles were counted before, and several days after application of Biscaya. Plant samples were collected 1-2, 7-9 and 14 days after application. Buds (> 2 mm) of the main stem and two side shoots were dissected to assess the number of eggs and larvae. New generation beetles where collected using soil-photoeclectors. In addition, greenhouse trials were done using untreated and insecticide-treated pollen beetles and plants collected from untreated and insecticide treated plots, respectively. Ten pollen beetles were caged on individual inflorescences using perforated plastic bags (10 replicates each). After 3-4 days the vitality of beetles was assessed and the buds dissected for eggs and larvae.

In all field trials the abundance of overwintered pollen beetles was reduced by Biscaya but only up to 7 days after application. The number of buds containing eggs and larvae was reduced significantly up to 14 days after application, with up to 86% lower infestation of buds compared to untreated plots. The abundance of emerging new generation beetles was reduced in Biscaya-treated plots up to 76%. In all greenhouse trials, the lowest number of infested buds was counted on Biscaya-treated plants. The results show that application of Biscaya at the bud stage (BBCH 53-55) is suitable for effective control of pyrethroid-resistant pollen beetles. It could form an important part of a resistance management program by causing lethal effects on pollen beetles as well as effects on egg laying and reproduction.

Key words: *Meligethes aeneus*, pyrethroid resistance, oviposition, thiacloprid, population dynamics

First data on RNAi to control pollen beetles

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Abstract: The pollen beetle, *Meligethes aeneus*, is one of the most economically important pests in European agriculture and is one of the most destructive pests of cruciferous oilseed crops. The resistance to pyrethroid insecticides in recent years in several EPPO countries has led to an increased need for novel pest control strategies such as RNA interfering (RNAi) for controlling the insect. The aim of this work is to evaluate the RNAi technique for investigating the functional genomics and its potential for controlling *M. aeneus*. The transcriptome data were deployed in order to find the candidate essential genes and subsequently, to design double-stranded RNA (dsRNA). To address the functionality of these essential genes, dsRNA was delivered to larvae by means of microinjection. Based on our preliminary data, the RNAi technique can be used as a novel method for controlling this important pest.

Key words: RNAi, *Meligethes aeneus*, pesticide resistance

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Pyrethroid sensitivity of adults and larvae of *Meligethes aeneus*

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Abstract: Control of pollen beetle (*Meligethes aeneus* F.) is complicated due to pyrethroid resistance of adults widely distributed throughout Europe. A high intensity of insecticide applications is common in spring because different insect pests occur over long periods as adult and larval stages in oilseed rape. In the past, pyrethroids were intensively used because of low costs and ease as they were often mixed prophylactically with fungicides. With each spring application, adult pollen beetles and larvae come into contact with insecticides, which selects for resistance. It is unclear clear if adults and larvae both express resistance. This study aimed to analyze the pyrethroid sensitivity of adults and their larvae from German, Romanian and Hungarian populations in 2015 and 2016.

Overwintered pollen beetles were collected in 2015 and 2016 at different locations in Romania, Hungary and northern Germany. The pyrethroid sensitivity of random samples of beetles was tested using the Adult-Vial-Test (AVT, IRAC Method 011) but with an assessment after 5 instead of 24 hours. Lambda-cyhalothrin (e.g. in Karate Zeon) was used. Different rates from 0.003 to 0.375 µg lambda-cyhalothrin/cm^2 were used with four replications each. To obtain pollen beetle larvae, oilseed rape was cultivated in the greenhouse at 15 °C and 15 beetles from the same batch were caged per plant and allowed to oviposit for 12-13 days. The species of caged adults was determined at the end of this period. The plants were kept at 15 °C in large saucers and the pots were covered with plastic bags to prevent larvae burrowing into the soil. Between 7-8 days after the egg laying period L2-larvae were ready to pupate and were collected in the saucers. Plants were fogged with water to enhance emigration of larvae. Larvae were inserted into coated glass vials similar to adults in the AVT. On the lid of the vials 2 µl of water was added to increase humidity. Vitality of larvae was assessed 5 hours after insertion. LD_{50}-values were calculated using POLO PLUS 2.0.

The species determination showed that all pollen beetles used in the study in 2015 were *M. aeneus*. In almost all cases larvae showed a similar response as the associated adults. In 2015 adults and larvae of German pollen beetles (LD_{50} 0.12/0.08 µg lambda-cyhalothrin/cm^2) showed an evidently lower sensitivity than those from Hungary (LD_{50} 0.03/0.05 µg lambda-cyhalothrin/cm^2), which again were less sensitive than Romanian beetles and larvae (LD_{50} 0.004/0.002 µg lambda-cyhalothrin/cm^2). Even at 0.375 µg lambda-cyhalothrin/cm^2 no total mortality was found in German beetles and larvae.

Key words: *Meligethes aeneus*, pyrethroid resistance, adults and larvae, lambda-cyhalothrin, Adult-Vial-Test

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Effects of conventional and dropleg insecticide application techniques on pests during flowering of oilseed rape

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Abstract: We conducted two years field experiments in Germany comparing conventional and dropleg spraying techniques. We found slightly reduced efficacy of dropleg spraying of Biscaya and Mavrik on oilseed rape at BBCH growth stage 65 (full flowering) against insect pests (*Meligethes aeneus, Ceutorhynchus pallidactylus, C. obstrictus* and *Dasineura brassicae*). The dropleg technique therefore seems suitable for control of insect pests during the flowering stage of oilseed rape, when exposure for bees and other pollinators needs to be minimized. When testing Biscaya and Mavrik in 2015, the latter had lower efficiency.

Key words: application technique, dropleg, insecticides, flowering stage, bees, efficacy

Introduction

In many occasions in winter oilseed rape cultivation the control of cabbage pod midge *Dasineura brassicae* or cabbage seedpod weevil *Ceutorhynchus obstrictus* is necessary during the flowering stage. Application of insecticides during flowering can result in exposure of pollinating insects such as honey bees, bumble bees and wild bees to insecticides. In addition, residues of insecticides in bee products such as honey have to be expected (Heinkel *et al.*, 2014). The pollinators may be adversely affected by direct and sublethal effects of the insecticides and pollination can be reduced because of repellency caused e.g. by pyrethroids. Alternative application techniques such as the dropleg system (Lechler, 2016) avoids contamination of the upper plant canopy layer and can reduce the risk for bees. However, the efficacy against target pests still needs to be comparable to conventional foliar sprays.

Material and methods

In 2014 and 2015 field experiments were carried out using conventional and dropleg spraying techniques with the same insecticides in plots (12 x 20 m) with 4 replicates. The insecticide Biscaya (72 g thiacloprid/ha) and in 2015 additionally Mavrik (48 g tau-fluvalinate/ha) were applied to the crop at full flowering (BBCH 65), both with 300 l water/ha and a driving speed of 7 km/h. The dropleg nozzles were spraying horizontally and downwards just below the canopy level with open oilseed rape flowers, so that only a minimum of open flowers were exposed to insecticides. The nozzles of the conventional technique were kept about 50 cm above the canopy level as usual.

To assess effects on insect pests, adult beetles were regularly counted on plants and adults and larvae dropping down to the soil were caught at regular intervals in trays (10 x 60 cm) filled with water were placed on the soil between the rows. Before harvest, the number of pods infested by *D.* brassicae and *C. obstrictus* was assessed and at BBCH 75 the number of larvae of *C. pallidactylus* in stems of oilseed rape was counted.

Results and discussion

Though pollen beetle usually do not need control at BBCH 65, when the application took place, the effects on the L2-larvae dropping to the soil is an indication of the efficacy of the products applied. The number of L2 dropping into the water filled trays in the control plots were about 3,000 per m² during the whole developmental period, whereas the number was reduced by about 70% in conventional Biscaya, by 41% in conventional Mavrik, 32% in dropleg Biscaya and not at all in dropleg Mavrik.

There were usually less *D. brassicae* or *C. obstrictus* infested pods in 2014 and 2015 (Figure 1 and Figure 2) with conventional applied insecticides, but in 2015 values for *C. obstrictus* were very similar between the techniques. Generally greater effects were observed for Biscaya compared to Mavrik.

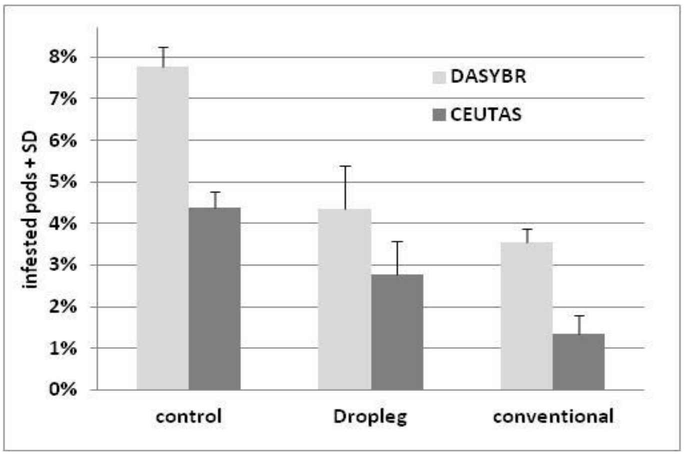

Figure 1. Pod infestation (mean + SD) by *Dasineura brassicae* (DASYBR) and *Ceutorhynchus obstrictus* (CEUTAS) in Wendhausen, Germany 2014 at BBCH 80 after treatment with Biscaya at BBCH 65 using conventional and dropleg spraying techniques.

Surprisingly, the application of insecticides at BBCH 65 reduced the number of *C. pallidactylus* larvae in stems (Figure 3). Though about 30% of larvae had already left the stems at the date of stem dissection (BBCH 75) a clear effect of Biscaya was visible with both application techniques, whereas Mavrik does not seem to have any effect. This difference may be explained by the systemic activity of thiacloprid in Biscaya or by the sublethal effects on oviposition as demonstrated by Brandes *et al*. (2016) for pollen beetles.

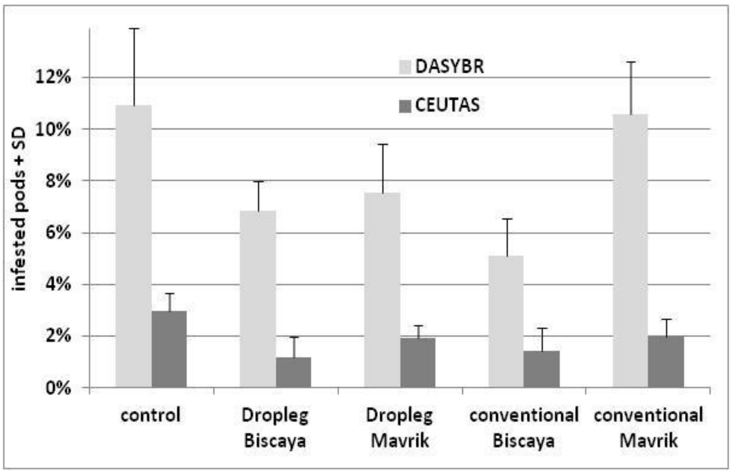

Figure 2. Pod infestation (mean + SD) by *Dasineura brassicae* (DASYBR) and *Ceutorhynchus obstrictus* (CEUTAS) in Wendhausen, Germany 2015 at BBCH 87 after treatment with Biscaya and Mavrik at BBCH 65 using conventional and dropleg spraying techniques.

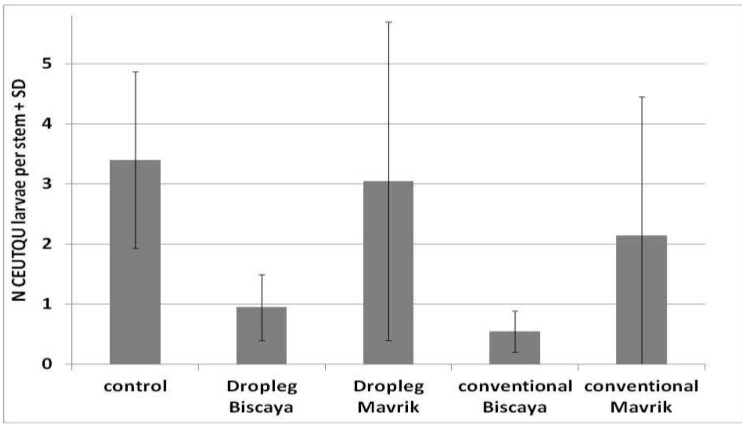

Figure 3. Stem infestation by larvae of *Ceutorhynchus pallidactylus* (CEUTQU) (mean + SD) at BBCH 75 in Wendhausen, Germany 2015 after treatment with Biscaya and Mavrik at BBCH 65 using conventional and dropleg spraying techniques.

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Implications of the neonicotinoid restriction on oilseed rape pest control, pollination and productivity

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Abstract: On the 1st of December 2013 the European commission restricted the use of three neonicotinoids (clothianidin, imidacloprid and thiamethoxam) as seed treatments on crops florally attractive to bees, including oilseed rape (OSR, *Brassica napus*) (European Commission 2013). The restriction was in response to concerns over the potential effects of these chemicals on non-target species, especially bees. Since the restriction, substantial research effort has focused on the impacts of neonicotinoids on bee species (e.g. Godfray *et al*., 2014 and Godfray *et al*., 2015). However, little attention has been given to understanding the effects of the restriction, and the impacts of alternative pest control methods, on OSR production and other non-target species such as natural enemies.

OSR is the third most widely grown crop in the UK, after winter wheat and barley, covering 653,000 hectares in 2015 (DEFRA, 2015) and with an average yearly value of £ 804 M (Nicholls 2016). It is an important break crop in a cereal rotation and provides an important temporal spike of floral resources for flower visiting insects. Since the restriction came into force, farmers in the south/east of England have been reporting severe crop damage primarily from attack by the cabbage stem flea beetle (*Psylliodes chrysocephala*), which affects around 67% of the OSR grown in the UK, and accounting for a loss of 3% of the national production (Wynn *et al*., 2014). There is also growing concern of a potential impact from turnip yellow virus (TuYV), transmitted by the peach-potato aphid (*Myzus persicae*) with 72% of winged females carrying the virus (Stevens *et al.*, 2008) which can cause yield losses up to 26% in the UK, pre restriction (Stevens *et al*., 2008). Little is known about how TuYV transmission will be impacted by the neonicotinoid restrictions. Both *P. chrysocephala* and *M. persicae* were controlled by the use of neonicotinoid seed dressings and subsequent pyrethroid foliar sprays in the autumn. Since the restriction it has been reported that the control threshold levels of five *P. chrysocephala* larvae per plant, has been exceeded in 46% of the planted crop (White, 2016). This has led to pyrethroid sprays being applied up to four times per crop in an attempt to control the beetles (White, 2016). However, both *P. chrysocephala* and *M. persicae* are known to be resistant to pyrethroids. *Psylliodes chrysocephala* is known to exhibit resistance in continental Europe (Højland *et al*., 2015) and in September 2014 resistance was recorded in the UK and has been shown to be present in 73% of the adults tested. The peach-potato aphid has also been known to be resistant to pyrethroids for a number of years (Devonshire & Moores, 1982) and may be capable of developing resistance to neonicotinoids (Bass *et al.*, 2011). Thus, the use of pyrethroids may be ineffective at controlling the pests, even increasing resistant populations, but could well be detrimental to the beneficial insect population. Given the restriction on neonicotinoid use, coupled with pyrethroid resistance and projections of milder winters in the UK, which will increase the risk of pest damage, the impact of both *P. chrysocephala* and TuYV on OSR production in the UK is expected to increase. It has already been suggested that 38,000 ha

extra OSR would have been planted if seed dressings were still available (White, 2016) highlighting the farmers response to the restriction.

To better understand the full implications of the neonicotinoid restriction, it is important to examine how alternative methods of pest control impact crop protection, non-target insects including pollinators and productivity. Field trials at Rothamsted Research will test a variety of past, present and future chemical, and non-chemical Integrated Pest Management (IPM) practices in winter OSR. These will include neonicotinoid seed dressings, pyrethroid sprays, trap cropping, nurse cropping, elevated sowing rates and winter cutting. Pest abundance and damage, natural enemy communities, flower production, pollinator communities and yield will be assessed. These data sets will allow the interactions of pest management practice, yield and impact on and from beneficial insects to be quantified in winter OSR. The ultimate goal of this project is to run a cost: benefit analysis on multiple IPM tactics in winter OSR to incorporate pest management, ecosystem service provision and crop productivity. This will not only highlight the impacts of different pest management approaches on crop yield but also explore the implications for ecosystem service provision and farmer profits.

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Winter oilseed rape and honey bee colony losses in winter: is there a relationship?

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Abstract: Winter oilseed rape (*Brassica napus* L.) is a host plant for numerous pest insects. Pests are preferentially controlled by agronomic methods. However, when those methods are insufficient to prevent a surpassing of the economic damage threshold, insecticides are applied. Because of potential side-effects on beneficial organisms like honey bees (*Apis mellifera* L.), exposure of pollinators to insecticides should of possible be prevented by the method of application. It is a matter of dispute whether or not the prevention of exposure, particularly of pollinators, to neonicotinoid insecticides is sufficiently effective under field conditions. Here, we tested if the area covered by oilseed rape, the distance between the nearest oilseed rape crop and the size of the biggest oilseed rape crop in radii of 2 and 5 km around > 150 apiaries in Luxembourg were correlated (Pearson, $P < 0.05$) with winter honey bee colony losses (% per apiary). In the period 2010-2012, when neonicotinoid use was permitted and widespread as both spray application or as seed coating, no significant correlations were detected between oilseed rape land use parameters and honey bee colony losses in the winter following oilseed rape harvest – except for 2012 in northern Luxembourg, where the size of the biggest oilseed rape crop was positively related with honey bee colony losses within the 5 km radius around the apiaries. This individual observation was, however, neither reproducible in space, nor in time, so far. Our study thus provided no reproducible evidence for an effect of winter oilseed rape on honey bee colony winter losses in a region and period where neonicotinoid use was widespread.

Key words: *Apis mellifera*, colony losses, pollinator, land use parameter

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Entomology Session 2 –
Improving decision support for IPM

Would more data on the population dynamics of insect pests in oilseed rape support better decision support in IPM?

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Abstract: A better understanding of pest biology can lead to improved methods for integrated pest control. Until now there is limited knowledge on pest population multiplication, though insect pest numbers attacking crops in the following season are important for future pest pressure predictions and improved IPM. The number of next generation beetles in winter oilseed rape can be collected using ground photoeclectors (Figure 1). This method was used in 44 fields in Germany 2015 and nearly 100% of the next generation beetles of *Meligethes aeneus, Psylliodes chrysocephala, Ceutorhynchus obstrictus, C. pallidactylus* and *C. picitarsis* hatched from the soil within the eclectors were caught if the eclectors were kept in the field from BBCH 75-78 until harvest. Average/max. number of beetles caught per m² were: *M. aeneus* 212/3000, *P. chrysocephala* 298/2000, *C. obstrictus* 88/637, for *C. pallidactylus* 25/152, and for *C. picitarsis* 7/12 (only detected in South Germany at 3 locations). For *M. aeneus* the pest pressure in 2016 was limited in accordance with the low number of new generation beetles found in 2015. Population density of most species was quite evenly distributed within Germany but for *C. obstrictus* high densities occurred throughout the north and east of Germany, finally resulting in high pressure in this region in May 2016. Such monitoring data could give hints for areas with high pest attack in the next season, although winter or aestivation mortality still needs consideration. The data can also address questions on sustainable effects of insecticides and will lead to a better understanding of pest biology.

Figure 1. Ground photoeclector. The base ring (0.25 m²) is dug into the soil just after winter without disturbing the crop plants within and outside the ring and the tent is fitted at about BBCH 75-78, leaving all plant material grown inside the ring by bending it down before fitting the tent. The eclectors were then left until harvest date. At the top a trapping box or, for live insect catch, a perforated plastic bag, is fixed to catch all insects escaping through the opening supplying light. For pests such as *Dasyneura brassicae* and *C. napi* such traps would have to be established in the crops following oilseed rape.

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Climatic factors help predict stem weevil abundance

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Abstract: Biological and ecological features of the rape stem weevil (*Ceutorhynchus napi*) and cabbage stem weevil (*Ceutorhynchus pallidactylus*) are similar and they are often presented as a complex, although life cycles have important differences and demand different approach in their control. The critical point to determine the optimal time for insecticide treatment is the time of their migration into winter oilseed rape crops. Based on climatic factors various phenological forecasting systems have been developed and help producers to predict the date of pest immigration into crops. During four growing seasons, adult forms of *C. napi* and *C. pallidactylus* were trapped using yellow water traps at six locations within five Croatian counties with intensive oilseed rape production. The content of each trap was emptied once a week. Climatic data were obtained from the Croatian Meteorological and Hydrological Service for each year of the investigation and for each location. Exploratory data analysis, using regression tree analyses was done in R 2.30, applying the package "tree". Factors that can help in prediction of appearance and abundance of *C. napi* are: the growth stage of oilseed rape plants, daily sunshine hours and mean daily air temperature; for prediction of *C. pallidactylus* an additional important climatic factor was mean daily precipitation. Climatic factors are very important for migration of stem mining weevils from their hibernation places to oilseed rape fields and together with the growth stage of the crop are very useful in predicting the appearance of adult forms.

Key words: rape stem weevil, cabbage stem weevil, prediction, abundance, climatic factors

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Too hot to handle? – Impact of winter temperature on populations of stem-mining pest insects

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Abstract: Winter oilseed rape (*Brassica napus* L.) (WOSR) is an important host plant for numerous pests throughout the entire growing season. The cabbage stem weevil, *Ceutorhynchus pallidactylus* (Mrsh.) (Col.: Curculionidae), is commonly found in WOSR throughout Europe. The larvae of this species mine within the petioles of plants in spring time, resulting in yield losses up to 20%. As well as knowledge about the migration activity of this pest, information on the population size can be helpful in decision support; helping farmers to optimize pesticide applications. Eickermann *et al*. (2015) published a model based on long-term multi-site datasets from field observations using yellow water traps. The monitoring was conducted from 2007-2012 on 5 locations in Luxembourg. The developed model estimates if the economic threshold of *C. pallidactylus* (> 10 individuals per trap within 3 consecutive days) will be breeched in March, in relation to the mean winter air temperature in different periods in previous February. If mean winter temperatures between 5 and 13 February are closer to 4.0 °C than to -2.4 °C, no breach of the control threshold is to be expected in the subsequent March. The model allowed a forecast with an accuracy of 81.5% and was validated in the years 2013 to 2016 for the area of Luxembourg.

Our presentation will detail validation results and will discuss how winter air temperature influences the population of *C. pallidactylus* in its overwintering habitats. Using this model can be a first step *i)* to allow forecasts about the population density of this specific pest species and *ii)* to develop additional forecast algorithms for pest species which are sharing a similar ecological niche, like the pollen beetle, *Meligethes aeneus*. Being aware of the estimated number of pest individuals, the monitoring effort by farmers might be saved in years with high winter temperatures and the number of chemical applications for controlling *C. pallidactylus* could be reduced.

Key words: crop invasion, decision support tool, DSS, oilseed rape, overwintering habitat

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How can decision support system forecasts improve management of pollen beetle and cabbage stem flea beetle in oilseed rape?

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Abstract: Risk assessment for insect pests in oilseed rape (OSR; *Brassica napus* L.) is complex as the crop is attacked by a suite of different insect pests at almost every stage in its growth. Treatment thresholds (an abundance of pests beyond which economic losses may result) are available for most insect pests in OSR but in most cases monitoring to determine threshold breaches is time consuming and therefore onerous, resulting in many growers deciding to treat prophylactically.

Over-use of cheap and effective insecticides has led to heavy selection pressure for resistance and pyrethroid-resistant pests are now widespread across Europe (e.g. Thieme *et al*., 2010; and papers in this volume: Heimbach & Brandes, 2016; Robert *et al*., 2016; Seidenglanz *et al*., 2016; Brandes & Heimbach, 2016). To reduce further selection pressure and to aid optimal and judicious use of the more expensive active ingredients, on-line decision support system (DSS) tools driven by local meteorological data can help to focus monitoring effort to when it is most needed. We assessed the proPlant.expert DSS (Newe *et al*., 2003) for pollen beetles (*Meligethes aeneus*) in the UK and found that using it could almost half monitoring effort compared with following conventional monitoring advice (Ferguson *et al*., 2015). We describe how a free version of this, the Bayer Pollen Beetle Predictor, available to growers in the UK can be used to reduce monitoring effort, save unnecessary prophylactic sprays and help time spray application.

We also describe the devastating effects of the cabbage stem flea beetle (*Psylliodes chrysocephala*) on OSR production in some areas of the UK since the revocation of neonicotinoid seed treatments (e.g. Coston *et al*., 2016) and discuss how DSS tools such as proPlant could be used to help predict migration to the crop and time more effectively sprays against the larval stages.

Key words: immigration, migration, crop invasion, DSS, oilseed rape, overwintering habitat

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High Performing Computer as an efficient tool for forecasting pest insect activity

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Key words: crop invasion, decision support tool, DSS, oilseed rape

Introduction

The pollen beetle, *Meligethes aeneus* (Fabricius), is a common pest insect in winter oilseed rape (WOSR) in Europe and responsible for possible yield losses of up to 80% (Hansen, 2003). The extent of field losses depends on meteorological conditions and the number of pesticide applications that should be kept to a minimum. Therefore, knowledge concerning the date of the crop invasion by pests is crucial for the timing of chemical controls. Crop invasion by the pollen beetle is driven by different meteorological variables e.g. air temperature, soil temperature, sunshine duration, wind speed and precipitation (Ferguson *et al*., 2014; Junk *et al*., 2015). Appropriate decision support systems (DSS) that take into account all relevant meteorological variables should be able to forecast the first invasion of pest species with a high level of accuracy. Based on multi-year field observations of the pollen beetle migration at five different test sites in Luxembourg, we were able to develop a phenological model to forecast the first crop invasion by *M. aeneus* in spring. The setup of those DSS is time consuming and requires high computational resources. Therefore, a high performance computer is a suitable tool for the development of such DSS.

Material and methods

The dataset to develop the phenological model consists of field observations of the date of first activity of *M. aeneus* together with local meteorological measurements. During the period from 2008 until 2013, data were obtained from five field sites in Luxembourg: Burmerange, Christnach, Obercorn, Reuler and Useldange. At each site, immigrating individuals of *M. aeneus* were caught with yellow water traps (four per site, three times per week between February and June) based on Williams (2010). Meteorological data were obtained from automatic weather stations located directly beside each of the five test fields. Daily values of air temperature, mean soil temperature (0.05 m depth), precipitation and sunshine duration were retrieved and pre-processed via an automatic data processing chain for gap detection, quality and plausibility checking. A threshold-based model for predicting migration of *M. aeneus* into WOSR was developed. To optimize computational resources, plausible ranges of the meteorological variables were defined for the later analysis: mean air temperature 0-30 °C; mean soil temperature 0-20 °C; sunshine duration 0.1-12 h. The method for determination of the final threshold values is shown in Figure 1. All possible combinations of air temperature, soil temperature and sunshine duration, with increments of 0.1 were compared to the time series of the observed meteorological data at the respective field. Combinations that did not exist in the measured data sets were excluded from further analysis. The day of year (DoY) when the combination of meteorological variables first occurred in the measured time-series was identified and compared with the date of first crop invasion using the root mean squared error (RMSE) as quality criterion. Precipitation was included because it hinders flight activity and threshold values of 0.2, 0.5, 1.0, 2.0, 3.5, or 5.0 mm (daily totals) were tested. In addition, the persistence of two and three consecutive days of all threshold values was assessed.

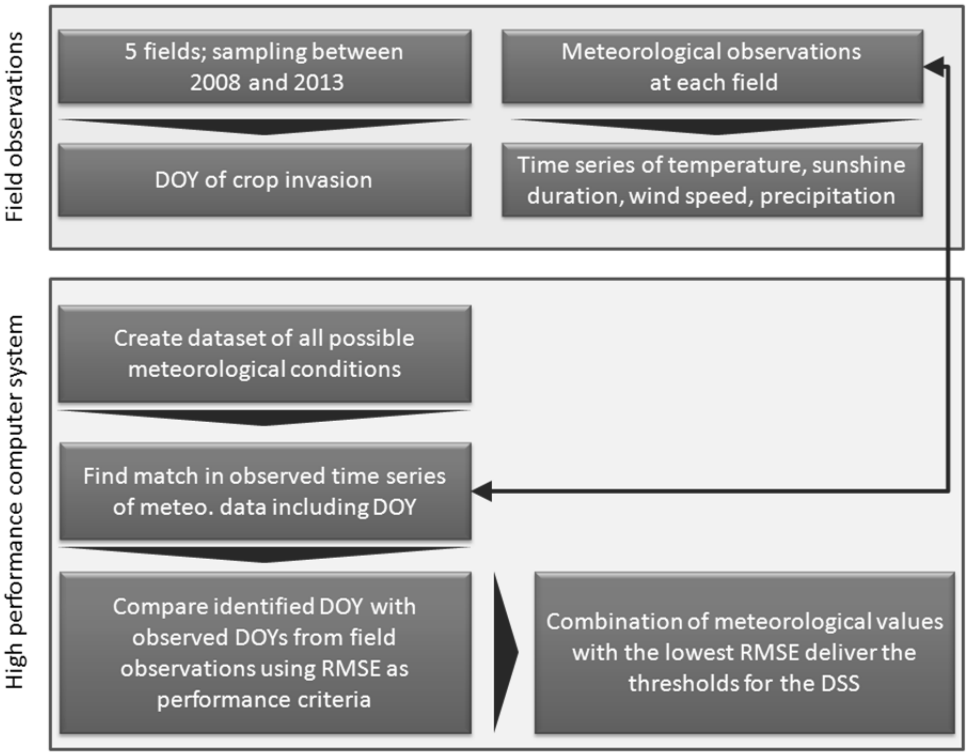

Figure 1. Description of the method to determine the optimal threshold values of meteorological variables for the decision support system based on multi-year field observations.

Results and discussion

The following thresholds were identified based on the quality criterion RMSE: mean air temperature 8.0 °C; mean soil temperature 4.6 °C; and sunshine duration 3.4 h. In addition, a value of 1.0 mm for precipitation and a persistence of one day showed optimum results. The RMSE for the model is 9.3 days, and approximately 4 x 10^9 model iterations were necessary to achieve this result. Differences between predicted and observed crop invasion was greater than 5 days in only six out of 30 cases. Currently the model is integrated in an operational agricultural warning system in the Grand-Duchy of Luxembourg. It should improve the pest management by provision of accurate warnings of crop invasion. In addition, the model can be easily transferred to other regions by adjusting the meteorological threshold values.

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Adults of *Psylliodes chrysocephala* in different types of yellow water traps in winter oilseed rape

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Abstract: Yellow water traps are a decisive factor in the forecasting system for *Psylliodes chrysocephala*, the Cabbage stem flea beetle. In the northern parts of Germany a threshold of 50 adults per yellow water trap (YWT) within three weeks is established. In Germany two types of yellow traps are widely used; a rectangular (26 x 33 cm) and a round type (22.4 cm diameter), both about 8 cm high. The use of a grid at the top of the YWT is recommended to keep out bees. The aim of this study was to investigate effects of different types of YWT with and without grid placed on the soil surface or dug into the soil on Cabbage stem flea beetle.

In autumn 2015 YWT in 5 variants (AG: angular with grid; A: angular without grid; RG: round with grid; R: round without grid; RD: round dug into the ground without grid) with at least 2 replicates were placed in 17 different winter oilseed rape fields all over Germany. The traps were filled with a 10% benzoic acid water solution with detergent with about 5 m distance between each trap on 1 side of a field. The grids used had a mesh size of 7 mm and were supplied together with the traps. Rectangular traps were sponsored by Syngenta and round traps by BayerCropscience.

Clearly more beetles are caught in the rectangular type of YWT compared to the round traps (Table 1). Taking the surface area of the traps into account (rectangular 858 cm^2, round 394.1 cm^2) the round ones catch more per cm² than the rectangular ones. With grids, the numbers are only slightly reduced. The YWT dug into the soil has the highest numbers which indicates that beetles might mostly get caught by walking or jumping into traps. The grids are a useful tool to protect bees of different species (Table 2), reducing the number of captured bees and bumblebees by 68.8% and 85.9 %, respectively. The study shows clearly that there may have to be variable thresholds for different YWT-systems.

Table 1. Mean percentage of total catch in different tallow water traps (± SD) at 17 field sites with at least 150 beetles per site (58 replicates altogether); n = 16332 beetles captured in September and October, 2015. (AG: angular with grid; A: angular without grid; RG: round with grid; R: round without grid; RD: round dug into the ground without grid).

YWT-Type	AG	A	RG	R	RD
% of total	20.6 ± 4.6	23.3 ± 5.3	14.1 ± 3.7	15.6 ± 2.6	26.5 ± 5.7

Table 2. Influence of the grid on the total number of bees (n = 567) and bumblebees (n = 6) in different types of yellow water traps at 34 field sites; 124 replicates of each trap-type. (AG: angular with grid; A: angular without grid; RG: round with grid; R: round without grid; RD: round dug into the ground without grid).

YWT-Type	AG	A	RG	R	RD
Bees	87	233	48	199	129
Bumblebees	6	48	2	9	18

Key words: Yellow water trap, cabbage stem flea beetle, bees

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Spatio-temporal distribution and association of cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsham, 1802) and pollen beetle (*Meligethes aeneus* Fabricius, 1775) in winter oilseed rape

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Abstract: The within-field spatio-temporal distributions and relationships of *Ceutorhynchus pallidactylus* (Marsham) (Coleoptera: Curculionidae) and *Meligethes aeneus* (Fabricius) (Coleoptera: Nitidulidae) in a crop of winter oilseed rape [*Brassica napus* L. (Brassicaceae)] were assessed in the Olomouc region (northern Moravia, Czech Republic) over the course of 2013-2015. Distributions were analysed using SADIE analysis (Spatial Analysis by Distance IndicEs), for associations we used Quick association analysis. In 2013, males and females of *C. pallidactylus* were within-field associated during the period of higher flight activity when beetles were rather edge distributed. The similar character of distribution patterns and significant association of both sexes of *C. pallidactylus* was recorded in mid-April 2015. Despite a random distribution of females of *C. pallidactylus* in the crop on April 22 2013 there was a significant spatial association between the adults of *M. aeneus* and the females that date. Significant association between non-randomly distributed females of *C. pallidactylus* and adults of *M. aeneus* was recorded on 21 April in 2015. In all years, adults of *M. aeneus* showed stronger tendency to aggregation in crops mostly during their higher flight activity. When populations of the assessed insects were non-randomly distributed in crops ($I_a > 1$ for $p < 0.05$), they were usually aggregated into one major and one adjacent patch clusters localized along the edge of field with tendencies to spread to central parts of crop, too.

Key words: SADIE analysis, spatial distribution and association, *Ceutorhynchus pallidactylus*, *Meligethes aeneus*

Introduction

The oilseed rape (*Brassica napus* L.; Brassicaceae) crop is host to a diverse community of invertebrates, including many pest and beneficial species (Alford *et al*., 2003). For future advances in integrated pest management (IPM) strategies including improved risk assessment and for the possibility of improved targeting of biological control, more understanding is necessary about the distribution and within-field spatio-temporal interactions of the main pests and their naturally occurring biological control agents (natural enemies) (Warner *et al*., 2000; Ferguson *et al*., 2006).

The spatial relationships between insects and crop plants remain an aspect of pest/host-plant ecology which has received insufficient attention. There is a lack of detailed information on the spatial heterogeneity of pest populations in oilseed rape at the crop scale, and the effect of this heterogeneity on a crop yield and on implications for sampling decision-making in integrated pest management (IPM). Spatial information is also needed for studies of insect movements into and within crops (Murchie *et al*., 1999) to underpin the development of pest control strategies in which application of pesticides may be spatially targeted to minimize their negative impact on beneficial insects (Evans & Stafford *et al*., 1998). The spatial distribution pattern for an insect species has, in the past, often been measured using the relationship between the variance and mean (Taylor, 1984, Clark & Perry, 1994) and association between species has been measured by the correlation coefficient. These approaches do not utilize information about the location of insect counts. In the past decade, the development of novel spatial statistics, notably Spatial Analysis by Distance IndicES (SADIE, Perry, 1998 a; b) has enabled the spatial information in a two dimensional array of sample counts to be used as a part of the analysis, giving a much more detailed and informative picture of the pattern of crop colonization by insect species. The method has enabled any association between different species to be analysed (Williams & Ferguson, 2010; Ferguson *et al*., 2000).

The spatial distribution of insect pests infesting oilseed rape was one subject of studies by Free & Williams (e.g. Free & Williams, 1978; 1979 a; b). Insects were sampled along straight-line transect into crops, and from discrete points at the edges and in the centres of crops to determine pest distribution during their immigration into and infestation of the crop (Warner *et al*., 2000).

The first objective of this paper is to describe the spatial distributions of cabbage stem weevil (*Ceutorhynchus pallidactylus* Marsham, 1802; Coleoptera: Curculionidae) and pollen beetle (*Meligethes aeneus* Fabricius, 1775; Coleoptera: Nitidulidae) in winter oilseed rape using SADIE analysis. The second objective of this paper is to investigate if there are some spatial associations between the pollen beetles and both sexes of cabbage stem weevils (interspecific association) and also to compare the intraspecific relationships in the distribution of males and females of cabbage stem weevil by Quick association analysis.

Material and methods

Crop site and insect sampling

Winter oilseed rape crops located in the Olomouc region (northern part of Moravia, Czech Republic) were sampled 2013-2015. No insecticides were applied to the crops in all three years. On the crops (2013: 1.4 ha, 2014: 0.7 ha, 2015: 0.8 ha) insect samples were collected using yellow water traps (YWT) (Möricke's dishes) installed across the crops in a rectangular grid (6 × 6 = 36 places in 2013, 5 × 5 = 25 places in 2014 and 2015). Each YWT was characterized by its y and x coordinates. The distance between a field edge and the nearest YWT was 10 meters in all cases. YWTs were filled with water and several drops of detergent were added to break the surface tension in order to drown the insects. YWTs were emptied twice a week. The catches were preserved in bottles filled with 70% ethyl alcohol until they were sorted. In the laboratory species and sex determination of the captured insects was carried out. For distinguishing females from males of *C. pallidactylus* the previously published method (Seidenglanz *et al*., 2013) was used.

Analysis of pest distribution and association

Spatial distributions of adults of *C. pallidactylus* and *M. aeneus* were analysed using SADIE (Perry, 1995; 1998a; b). This technique enables the spatial characteristic of observed distributions to be assessed and compared by randomisation procedures, using indices and test of randomness. To describe the spatial pattern in single sets of 36 counts (25 counts in 2014 and 2015) of adult insect caught in YWT, two indices were used, the main SADIE index I_a and one subsidiary index J_a. In the case of non-random distribution of individuals of the tested population in crops, indicating a significant tendency to aggregate in clusters (patch or gap clusters) the value of I_a is higher than unity ($I_a > 1$ for $p < 0.05$). The index J_a discriminates between patterns, where there is one major cluster, for which its value is greater than unity, and two or more clusters, for which its value is equal to or less than unity ($J_a \approx 1$ for $p < 0.05$). SadieShell software version 2.0 was used for computing the indices. To assess association in distributions of two populations (♂ × ♀ of cabbage stem weevil and ♂, ♀ of cabbage stem weevil × pollen beetle) Quick Association analysis was used (version 2.0). The software calculates an overall spatial association index (X), based on the similarity of the local clustering indices previously stated for the two compared populations (Ferguson *et al*., 2006). Values of X are > 0 for distributions that are associated (for $p < 0.025$), around zero for distributions positioned at random with respect to another, and < 0 for distributions that are dissociated ($p > 0.975$). The mean number of pests caught in YWT and the standard error of the mean (SE) were calculated using standard routine statistical methods. For graphic visualisation of SADIE results Statistica software v. 12 (STATSOFT, Inc., 1984-2015) was used.

Results

Spatio-temporal distribution and association of C. pallidactylus in 2013

A total of 8270 adults of *C. pallidactylus* (of which 25.16% were females) were caught in the 36 YWT from April 8 to May 3. Only a few individuals were caught thereafter (the catches made after May 3 were not included in SADIE analysis). The major part of flight activity lasted for two weeks (from 15 to 30 April) and the relatively high catches were recorded on April 15 and 18 (for females also on April 25). On these dates (April 15, 18), significant overall spatial associations (X = 0.660; 0.861 for $p < 0.025$) between the *C. pallidactylus* sexes were recorded. The peak of female´s flight activity (mean: 25.13 individuals per trap) was recorded on the same day as for males (mean: 92.33 individuals per trap), on April 18. Then the catches continuously declined up to May 3 (0.02 ♂ and 0.00 ♀). Analysis by SADIE of the distribution of males indicated strong aggregation ($I_a > 1$ for $p < 0.05$) on April 15, 18 and 22. Females were significantly aggregated for four dates: April 15, 18, 25 and 30 (Table 1). On April 18 males and females were aggregated into two patch clusters localized in the north side and east side of the field. The gap cluster was localized on the south-west of the field ($I_a > 1$ and $J_a \approx 1$ for $p < 0.05$; Figure 1 A, B, Table 1). Females showed aggregation into more than one cluster on April 25, as well (Table 1).

Spatio-temporal distribution and association of C. pallidactylus in 2014 and 2015

A total of 339 adults of *C. pallidactylus* (of which 35.10% were females) were caught in 25 YWT from March 3 to April 11, 2014. In 2015, a total of 1313 adults (of which 59.86% were females) were caught from March 23 to June 6.

In 2014, males were randomly distributed within the field (I_a for $p > 0.05$) during the whole period of monitoring (March, April). Females showed significant aggregation only on April 7. The highest catches for both sexes were recorded on March 13 and April 1, but relatively low numbers of individuals occurred in YWT (2.24 ♂, 1.20 ♀ and 2.96 ♂, 1.24 ♀, respectively).

Table 1. Results of flight activity monitoring and SADIE for adults of *C. pallidactylus* and *M. aeneus*: Trap catches and aggregation indices characterizing the spatio-temporal distributions of the two pests monitored through YWT 2013-2015 (36 traps/2013; 25 traps/2014 and 2015).

Date of sampling	No. of *Ceutorhynchus pallidactylus*		No. of *Meligethes aeneus*	SADIE index *Cetorhynchus pallidactylus*				SADIE index *Meligethes aeneus*	
	Mean per trap (SE) ♂	♀	Mean per trap (SE)	I_a[1] ♂	J_a[2] ♂	I_a[1] ♀	J_a[2] ♀	I_a[1]	J_a[2]
8.4.2013	0.02 (0,02)	0.02 (0.02)	×	×	×	×	×	×	×
11.4.2013	0.13 (0.05)	×	0.02 (0.02)	×	×	×	×	×	×
15.4.2013	35.27 (3.68)	7.38 (0,90)	8.19 (0.58)	**2.20***	1.01	**2.01***	1.01	0.89	1.01
18.4.2013	92.33 (6.69)	25.13 (2.00)	109.97 (6.93)	**1.53***	**1.05***	**1.54***	**1,07***	0.92	0.98
22.4.2013	19.19 (1.24)	4.58 (0.68)	411.33 (28.27)	**1.41***	0.97	1.19	1.01	**1.77***	1.05
25.4.2013	14.33 (1.69)	10.91 (1.07)	433.63 (22.87)	1.05	1.09	**1.33***	**1.11***	1.30	1,00
30.4.2013	5.36 (0.63)	3.83 (0.38)	440.00 (21.81)	0.95	0.93	**1.45***	0.99	1.30	0.99
3.5.2013	0.02 (0.02)	×	359.97 (30.73)	1.20	1.08	0.97	1.05	**1.50***	1.03
3.3.2014	0,96 (0.17)	0.20 (0.10)	×	×	×	×	×	×	×
7.3.2014	0.16 (0.07)	0.12 (0.06)	0.08 (0.05)	×	×	×	×	×	×
10.3.2014	0.64 (0.17)	0.04 (0.04)	0.08 (0.05)	×	×	×	×	×	×
13.3.2014	2.24 (0.21)	1.20 (0.23)	0.12 (0.06)	1.09	1.01	1.18	1.00	×	×
17.3.2014	0.24 (0.16)	×	0.52 (0.15)	×	×	×	×	**1.36***	1.17
20.3.2014	0.48 (0.13)	0.16 (0.07)	1.08 (0.27)	1.01	1.02	×	×	1.29	0.90
24.3.2014	0.56 (0.15)	0.44 (0.17)	14.48 (1.52)	0.90	0.80	0.79	0.79	**1.77***	0.90
27.3.2014	0.12 (0.06)	0.32 (0.14)	8.76 (1.36)	×	×	×	×	**1.91***	1.01
1.4.2014	2.96 (0.45)	1.24 (0.21)	59.64 (5.63)	1.04	0.92	0.95	0.92	**1.73***	0.94
3.4.2014	0.28 (0.09)	0.12 (0.06)	31.52 (3.53)	×	×	×	×	**1.87***	0.97
7.4.2014	1.00 (0.23)	0.92 (0.19)	149.88 (10.64)	1.31	0.99	**1.51***	1.03	1.14	1.04
11.4.2014	0.20 (0.10)	0.12 (0.06)	16.48 (1.46)	×	×	×	×	0.82	1.08
23.3.2015	0.28 (0.10)	×	×	×	×	×	×	×	×
26.3.2015	0.84 (0.20)	0.04 (0.04)	×	×	×	×	×	×	×
30.3.2015	0.04 (0.04)	×	×	×	×	×	×	×	×
13.4.2015	6.08 (0.86)	7.28 (0.88)	17.76 (1.99)	**1.64***	1.00	1.01	1.07	1.23	1.10
15.4.2015	3.08 (0.47)	5.04 (0.67)	1.20 (0.18)	**1.44***	**1.16***	**1.48***	**1.06***	0.89	1.08
21.4.2015	1.12 (0.24)	3.12 (0.42)	19.92 (2.75)	1.17	0.90	**1.56***	**1.04***	**1.75***	**1.03***
29.4.2015	7.16 (1,40)	13.40 (2.21)	115.20 (13.61)	1.14	1.19	0.94	1.06	**1.42***	**1.10***
1.5.2015	1.16 (0.34)	0.08 (0.05)	26.88 (3.67)	**1.35***	1.40	×	×	1.16	1.15
4.5.2015	0.20 (0.08)	0.12 (0.06)	90.04 (8.83)	×	×	×	×	**1.34***	**1.07***
7.5.2015	0.48 (0.14)	0.88 (0.20)	156.32 (13.43)	1.05	1.08	1.23	1.03	1.17	1.12
11.5.2015	0.48 (0.15)	0.92 (0.20)	165.72 (16.62)	1.20	0.99	1.08	1.12	1.24	1.10
14.5.2015	0.12 (0.06)	0.32 (0.14)	174.68 (13.41	×	×	×	×	**1.37***	1.04
18.5.2015	×	0.12 (0.08)	153.44 (15.75)	×	×	×	×	1.19	1.08
21.5.2015	×	×	90.08 (8.75)	×	×	×	×	**1.61***	1.01
25.5.2015	×	0.04 (0.04)	83.16 (6.58)	×	×	×	×	1.04	1.04
29.5.2015	0.04 (0,04)	0.04 (0,04)	88.00 (12.85)	×	×	×	×	**1.85***	1.08
3.6.2015	×	0.04 (0,04)	46.16 (5.30)	×	×	×	×	1.17	1.01

[1] Values of $I_a > 1$ indicate aggregation within the sample area (***bold** values $p < 0.05$)
[2] Values of $J_a \approx 1$ indicate the presence of multiple clusters when $I_a > 1$ (***bold** values $p < 0.05$)

In 2015, the major flight activity period for both sexes lasted roughly for two weeks (from April 13 to 29). Males were non-randomly distributed on two dates: April 15 and May 1 ($I_a > 1$ for $p < 0.05$). Females showed aggregation in one major patch cluster and to another two patch clusters ($I_a > 1$ for $p < 0.05$ and $J_a \approx 1$ for $p < 0.05$) situated along the eastern margin of the field April 15 and 21 (the situation from April 15 is displayed in Figure 1 D). On April

15, males showed aggregation in one major patch cluster and to another adjacent patch cluster (Figure 1 C). Both were localized in similar areas of the field as those for females. On the same date, both sexes were significantly spatiallyassociated (X = 0.475 for $p < 0,025$).

Spatio-temporal distribution of Meligethes aeneus and its spatial association with C. pallidactylus in OSR crops 2013-2015

The first adult of *Meligethes aeneus* was caught in YWT on April 11, 2013 (mean 0.02 adults per trap). Subsequently, numbers of beetles increased and very high catches were recorded from April 22 to April 30 (more than 400 adults caught per trap). Thereafter numbers of adults in traps slightly decreased (Table 1).

Spatial distribution proved to be non-random (I_a >1 for $p < 0.05$) on April 22 and May 3 2013, when beetles always showed aggregation in one major patch cluster (J_a for $p > 0.05$) localized along the south-eastern edge of the field (the situation from April 22 is shown in Figure 1 F). Despite the random distribution of females of *C. pallidactylus* in the crop on April 22, 2013, there was a significant spatial association between the pollen beetles and the females on that date (X = 0.420 for $p < 0.025$).

In 2014, the first pollen beetles were caught on March 7 (mean 0.08 adults per trap); the flight activity somewhat increased from March 24 to April 11, but in comparison with the previous and the next seasons the catches stayed relatively low during the duration of monitoring (Table 1). The distribution of *M. aeneus* adults in the crop was non-random ($I_a > 1$ for $p < 0.05$) on March 17 and March 24 to April 3. On these dates the beetles always showed significant aggregation in one major patch cluster (J_a for $p > 0.05$). On the day when the highest flight activity was recorded (April 7), the distribution of beetles in the crop was random (I_a for $p > 0.05$).

In 2015, the first beetles were caught on April 13 (mean 17.76 per trap). The first peak of flight activity (on average more than 100 pollen beetles per trap) was recorded on April 29. Relatively high flight activity (on average more than 150 individuals per trap) was recorded from May 7 to 18. According to SADIE results, distributions of *M. aeneus* were non-random (beetles were significantly aggregated) on April 21, 29 and on May 4, 14, 21 and 29 ($I_a > 1$ for $p < 0.05$, Table 1). The presence of two or more patch clusters ($J_a \approx 1$ for $p < 0.05$) was recorded on April 21 and 29 and on May 4 (the situation from April 21 2015 is shown in Figure 1 E). On April 21 significant overall spatial association (X = 0.675 for $p < 0,025$) between distributions of *M. aeneus* and *C. pallidactylus* females occurred in the crop.

Discussion

Our work supports previous reports that the numbers of insect pests (and also their natural enemies and non-target organisms in general) and their distribution in different zones of a crop often quickly change during the period of crop colonisation and the direction of the changes are affected by many environmental factors, including proximity of hibernation sites, field size and shape, structure of vegetation in and surrounding the field, weather conditions (direction of prevailing winds, temperatures, precipitation etc.), tillage system and pesticides used, etc. (e.g. Free & Williams, 1979 a; b; Hiiesaar & Metspalu *et al*., 2003; Ferguson *et al*., 2003 a; b). Williams & Ferguson (2010) described that rate of spread of females of *C. pallidactylus* into the crop from the edge is probably influenced by crop size and availability of suitable plants at the edge for egg-laying.

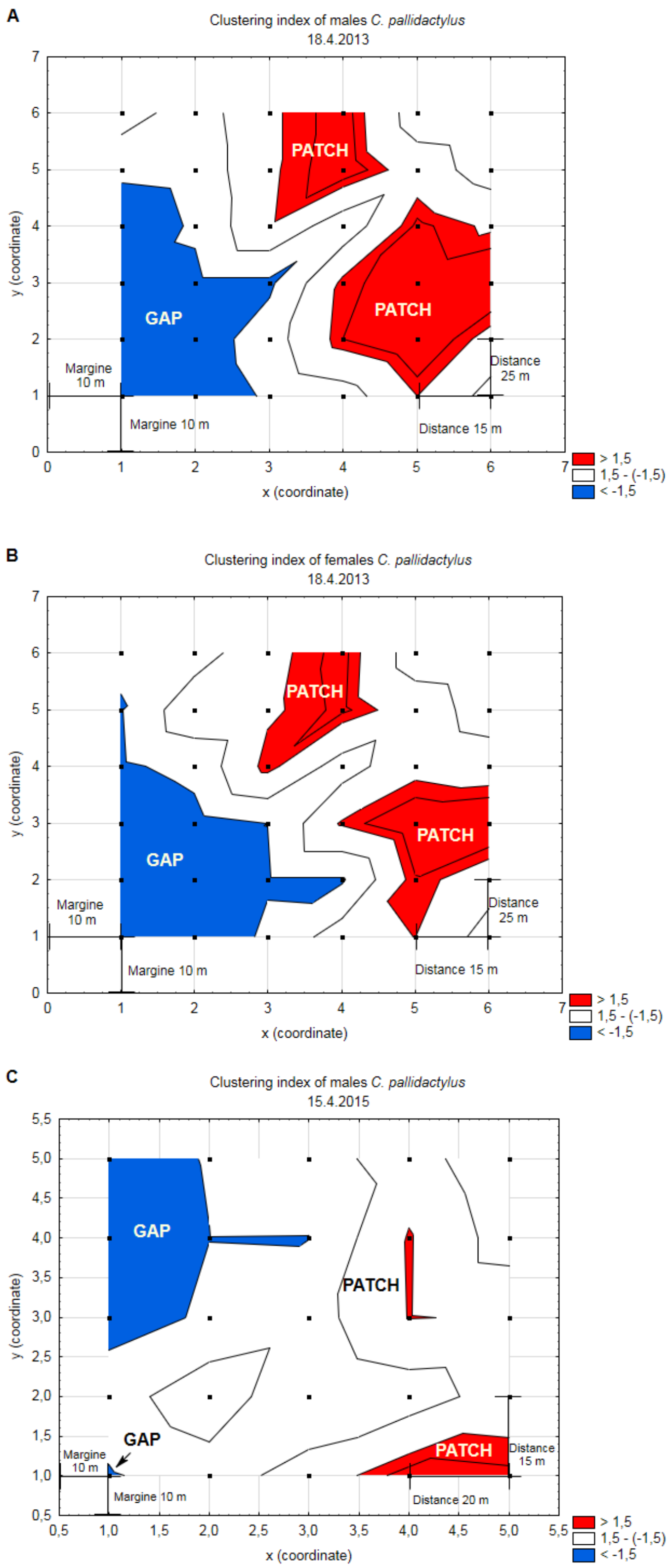

Figure 1. A-C. Graphical illustration of SADIE results for males and females of *C. pallidactylus* on April 18, 2013 (**A**, **B**) and on April 15, 2015 (**C**). Red (patch) and blue (gap) areas were identified by SADIE as patch and gap clusters in pest distributions. The black points mark the sampling places arranged in a rectangular grid (6 × 6 points: 36 sampling places in 2013 and 5 × 5 points: 25 sampling places in 2014 and 2015).

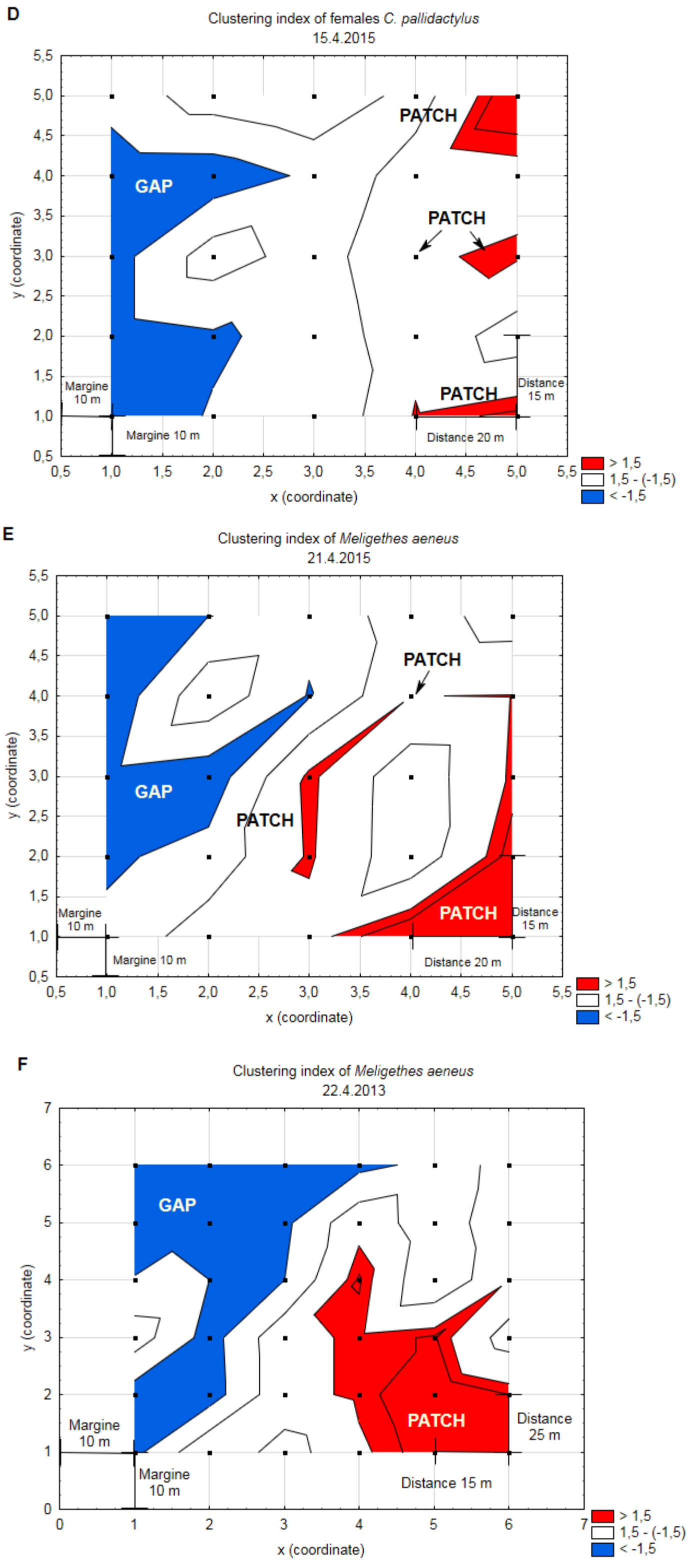

Figure 1 (continued). D-F. Graphical illustration of SADIE results for males and females of *C. pallidactylus* on April 15, 2015 (**D**) and for adults of *M. aeneus* on April 21, 2015 (**E**) and April 22, 2013 (**F**). Red (patch) and blue (gap) areas were identified by SADIE as patch and gap clusters in pest distributions. The black points mark the sampling places arranged in a rectangular grid (6 × 6 points: 36 sampling places in 2013 and 5 × 5 points: 25 sampling places in 2014 and 2015).

The knowledge of spatial and temporal changes in distribution patterns of insect pests can improve both timing and targeting of insecticide applications to the different zones of the crop according to pests densities. This may help to conserve the natural enemies of crop pests. The sampling of *C. pallidactylus* and *M. aeneus* at regular time intervals from points on a grid across a whole crop and the analysis of these data by SADIE have produced a much more detailed picture of the pattern of crop colonisation by these pests. The spatial distribution patterns of beetles within a winter oilseed rape crop indicated that infestation was greatest up to 30 m from the crop edge and the least in the central parts of crop. These data support studies published by Free & Williams (1978; 1979 a; b) and Williams & Ferguson (2010) which described differences in the spatial and temporal crop colonisation by *M. aeneus*. According to these authors, pollen beetles first infest plants at the edge of an oilseed rape crop and later spread towards crop centres after which the proportion at the edge diminishes. Klukowski (2006) investigated colonisation of winter oilseed rape crops by *C. pallidactylus* and found that males arrived before females, both were aggregated and more concentrated at the crop edge (compare with Figure 1 C, D) and the females tended to stay at the crop edge for longer than the males before spreading further into the crop centre during the period of egg-laying (Williams & Ferguson, 2010).

In this study, in 2013, males and females of *C. pallidactylus* were spatially associated in the crop during the time of their higher flight activity. The patch clusters stated for them were mainly localized along the edges with tendency to spread to central parts as previously reported (Figure 1 A, B). The similar character of distribution patterns and significant association in distributions of the both sexes of *C. pallidactylus* were clear on April 15, 2015, but in general it is not possible to conclude that the distributions of males and females of *C. pallidactylus* are spatially associated during the spring in the course of crop colonisation – due to variation in response with date (on some dates yes and on some dates no).

The distribution of females of *C. pallidactylus* was random four days after their highest flight activity in 2013 (Table 1), but, at the same time were spatially associated with non-randomly distributed beetles of *M. aeneus*. Significant association between non-randomly distributed females of *C. pallidactylus* and adults of *M. aeneus* was recorded on April 21 2015. In some periods of oilseed rape colonisation such a situation could occur that the populations of *C. pallidactylus* and *M. aeneus* adults show spatial association in their distribution. However, such situations did not occur regularly and probably they usually have only a short duration. On the other hand there were not any signs of dissociation between the distributions of pollen beetles and cabbage stem weevils in crops – pollen beetles probably do not show any tendency to elude the places already occupied by the cabbage stem weevils (adults).

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Automatic extraction of *Psylliodes chrysocephala* larvae versus sorting by hand

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Abstract: An easy but not widely used method for estimating the number of *P. chrysocephala* larvae was tested in this study. The Funnel-Method can be considered as a modified Berlese-Funnel-Method with no heat supply. The passive extraction of cabbage stem flea larvae by using a funnel and a catching vessel generated nearly the same results as the manual dissection. The big advantage of using the Funnel-Method is that distinctly less labour time is needed; the disadvantage is that the extraction needs about 21 days until all larvae have left the plant tissue and that larval development stages cannot be related to the collection date. The results of this study indicate that the Funnel-Method is a cheap and simple tool for practical use of estimating the total number of larvae per plant, but with a time delay before results are available.

Key words: *Psylliodes chrysocephala*, extraction, larvae, Funnel-Method

Introduction

Psylliodes chrysocephala (L.) (Coleoptera: Chrysomelidae), the cabbage stem flea beetle, is a key economic pest of winter oilseed rape (*Brassica napus*) (L.) (Cruciferae) crops in northern and central Europe (Kaufmann, 1941; Bonnemaison, 1965).

The common existing forecasting system relies on monitoring of adults by yellow water traps during the main crop invasion period and relating catch to a threshold for control to prevent larval damage. The action threshold in northern Germany for beetles caught in yellow water traps is 50 beetles in 3 weeks (Hoßfeld, 1993). The action threshold for larvae is 5 larvae per plant (Godan, 1950). The conventional method for determining the number of larvae in a plant is by manual dissection. This needs a lot of time, experience and specific equipment and is not practical for farmers’ use or for analysis of large samples. An extraction method is used in France (Anonymous, 2015). The aim of this study was to test if an extraction, which needs less time than the manual dissection of plants, is reliable.

Material and methods

The Funnel-Method can be considered as a modification by using not heat supply of the Berlese-Funnel-Method (Southwood & Henderson, 2000). It is a very simple construction made out of a funnel (20 cm diameter) and a collecting vessel. The plant samples were placed in the funnel and stored in a climate chamber at about 20 °C for 21 days. The drying of the plant tissue induces the larvae to leave the plant and search for new plants. During this process the larvae fall down into a conservation liquid and can be counted easily (Figure 1).

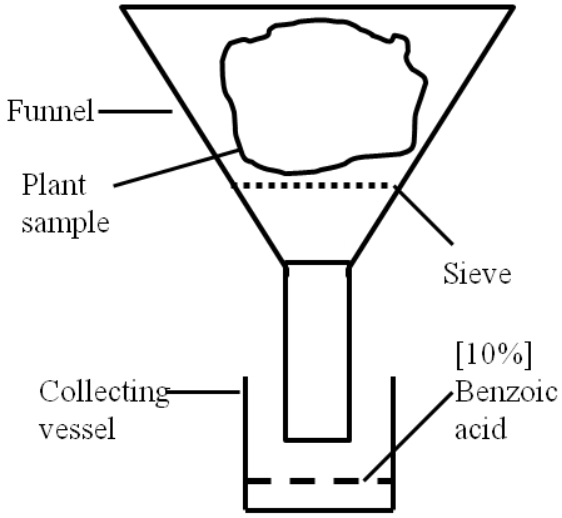

Figure 1. Schematic of the Funnel-System used to estimate the number of cabbage stem flea beetle larvae per plant.

To evaluate the efficiency of the method, samples of 80 OSR plants were taken without the root at random from a non-insecticide treated area (100 x 15 m) at a field site in Lower Saxony near Braunschweig. The samples were collected on 6 different dates in early spring (January-March 2016). To determine larval infestation by the conventional manual method, 20 plants were dissected from samples taken on every sampling date and 7 plants were placed per funnel using 6 replicates (in total 42 plants).

In a pilot test prior to the main experiment with plants sampled on January 5th 2016, the time was analyzed which is necessary to extract all larvae at an ambient temperature of about 20 °C (Figure 2). The trapping-vessel was emptied every third day and the number of larvae was counted. In this experiment after 21 days all larvae had left the plant tissue.

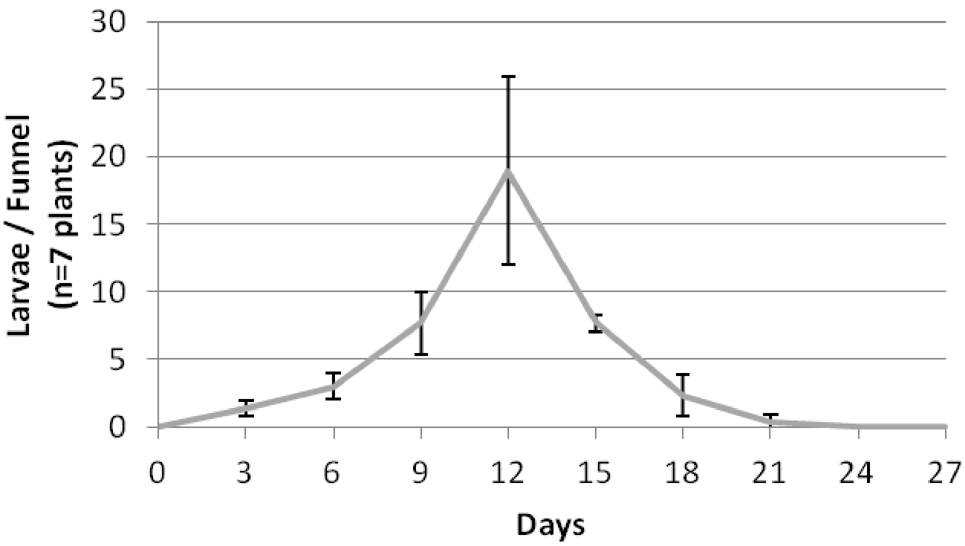

Figure 2. Mean larval number (±SD) extracted using the Funnel-Method over 4 weeks, 3 replicates.

The Funnel-Method was tested using samples collected on 6 dates with six replicates; each replicate consisted of one funnel loaded with 7 plants (BBCH 18-31) collected on each date. The main stem of the winter oilseed rape plants were cut in the middle, for faster drying. A sieve prevented the plants from falling into the collecting vessel, which was filled with 150 ml [10% in water] benzoic acid for a conservation of the larvae. The water tension was reduced by adding tensides (surfactant). The trapping systems were placed in a climate chamber at about 20 °C and a photoperiod of 12:12 (L:D) simulating laboratory conditions. The relative humidity was not controlled. After 3 weeks the vessels were emptied and the number of larvae was counted. The larvae were stored in a tray filled with 80% ethanol and the head capsule width measured later to determine larval instar stage.

Results and discussion

Figure 3 shows a higher heterogeneity in the larval infestations of the plants determined by manual dissection. This higher variability might be caused by using only 20 plants whereas 42 plants were used for the Funnel-method. The mean numbers of larvae per plant using the manual dissection and the extraction by the Funnel-Method are very similar for all sampling dates. But a big disadvantage of the funnel method is the time which is needed until all larvae have left the plant.

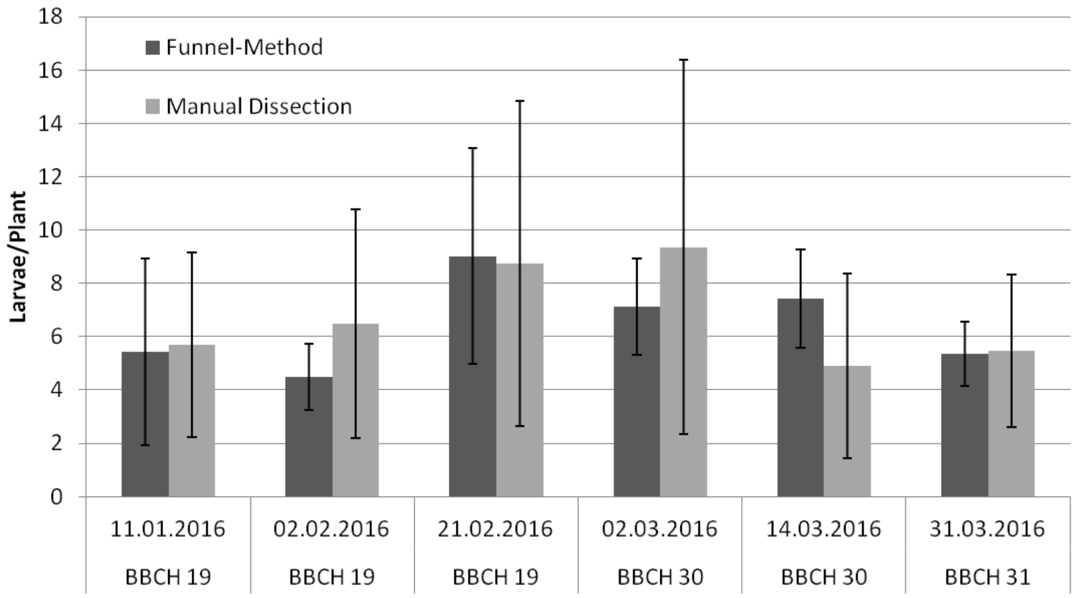

Figure 3. Number (± SD) of *P. chrysocephala* larvae per plant after 21 days extracted using the Funnel-Method (dark grey) with 7 plants per funnel and 6 replicates or manual dissection of 20 plants (light grey).

The larval instars were specified by measuring the head capsule using the classification after Kaufmann (1941). In samples taken on February 3rd 2016 about 98 % of the larvae (n = 227) extracted using the Funnel-Method grew up to the third instars (mean head capsule width of the L3 was 0.55 mm) before they left the plant. In contrast, the manual dissection of the same sample date showed a ratio of 34.2:49.7:6.1 (L1:L2:L3) with mean head capsule widths of 0.28:0.38:0.51 mm, respectively.

The measurement of the head capsule width showed that most larvae developed to the third instar stage before they left the plants. It seems that the larvae stay in the plants as long as is necessary or possible until they are ready to dig into the soil for pupation. The time needed for the development of a young L1 until leaving the plant for pupation at 20 °C is according to Kaufman (1941) 25 days. So our data are within a similar range.

It still has to be determined whether or not the method can also be used for larger plants than used in our experiment (plants at BBCH 18-31). The method can be used as a good tool in addition to plants dissections but cannot replace this traditional method fully. Any additional heat supply should be avoided to enhance the process because in preliminary experiments using such a system with heat supply only very small numbers of larvae were found. Therefore ambient temperatures higher than 20 °C should be avoided or the efficiency needs to be checked beforehand.

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Investigating the temporal and spatial ecology of the pollen beetle *Meligethes aeneus*

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Abstract: Oilseed rape (OSR) is the third most widely grown crop in the UK (> 600,000 ha; Defra, 2015) and is vulnerable to attack from the pollen beetle *Meligethes aeneus*. In cases of severe infestation levels yield can be reduced by 80% (Hansen, 2004). Pollen beetles colonise OSR plants during spring and cause feeding damage when the buds are developing. At this plant growth stage the adult beetles cause the main yield decreasing damage. Females lay eggs in the buds in which first instar larvae feed. Second instars feed on open flowers before dropping from the plant to pupate in the soil (Williams & Free 1978).

Current control methods rely heavily on the use of insecticides, but overuse and poor timing has led to the development of insecticide resistant populations of pollen beetles throughout Europe (reviewed in Thieme *et al*., 2010). Decision-support tools (such as proPlant) can be used in order to optimise use and reduce monitoring effort (Johnen *et al*., 2010; Ferguson *et al*., 2015). This tool is based local meteorological conditions and predicts migration events up to three days in advance. However, there is potential to use long-term spatially-explicit data to elucidate trends over time that could reveal other ecological mechanisms and thus improve predictions.

This project will utilise suction-traps (Taylor, 1962) and in-field samples (sticky traps, water traps and counts of beetles per plant) to model pollen beetle abundance both within-year across several sites in England and historically at two or more suction-trap sites.

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Entomology Session 3 – Delivering effective biocontrol of OSR pests via natural enemies

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Valuing natural pest control services for UK arable crops

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Introduction

Agriculture is a complex system that relies on various ecosystem services (Zhang *et al*., 2007). Natural pest control is an important regulating service, which can help to suppress pest damage and maintain crop yield (Power, 2010). However, the importance of natural pest control is often ignored by farmers worldwide (Grogan, 2014), and the knowledge base about the impacts of natural enemies is often lacking (Ekbom, 2010). How beneficial this ecosystem service is to crop production also depends on the pest management practices and pest damage levels in the field. Valuing an ecosystem service can reveal information on its roles in supporting human welfare, and also guide decision making on sustainable agriculture and environmental protection (UK NEA, 2011). However, limited valuation has been attempted for the natural pest control service (Letourneau *et al*., 2015), especially for UK arable crops.

The aim of this study is to quantify the natural pest control services for important UK arable crops, taking into account the influence of different pest management systems and pest density scenarios. Pests were arthropod pests for a specific arable crop, and natural enemies were related arthropod natural enemies. Arable crops included: wheat, barley, oats, oilseed rape, linseed, potato, sugar beet, field pea and field bean.

Material and methods

An online survey (via Qualtrics) was conducted within the Association of Independent Crop Consultants (AICC) to collect key information on natural pest control valuation: pest management systems for a specific crop (i.e. organic, integrated, and conventional), estimated reduced crop yields without arthropod natural enemies, and reduced insecticide treatment costs under integrated pest management systems (IPM). Information covered the average situation from 2010/11 to 2014/15. IPM is classified here as applying insecticide treatments according to arthropod pest densities in the field. Then economic surplus approach (Letourneau *et al*., 2015) was used to estimate the arthropod pest control services based on the weighted average information collected for each crop under different management systems. Organic systems were omitted due to lack of information. Three pest damage scenarios (low, medium, high) were incorporated in the calculations. Due to the availability of data, the evaluation was based on the UK crop harvests in the 2013/ 2014 season (DEFRA, 2015).

Results and discussion

For the 2013/14 season, the top three crops (out of nine) that benefited from arthropod natural enemies were wheat, barley, and potato.

Under the low pest damage level scenario, where arthropod pests would not cause damage to the crop yields even without natural enemies in the field, no values would be generated from natural pest control (Figure 1). Under the medium damage scenario, where pests would exceed the economic damage thresholds without natural enemies, both conventional and IPM systems would benefit from natural enemies by the increased crop yields, and IPMs also the reduced insecticide costs. For high pest densities, where natural enemies would not be able to suppress pests below the economic threshold, IPM users would not gain from the insecticide cost reductions, but increased crop yields would still apply for both systems. According to experts' opinions, natural enemies could also provide pest control services to conventional farmers.

Our study provides insights into valuing natural pest control services for UK arable crops. Limitations exist and further studies are needed for evaluations.

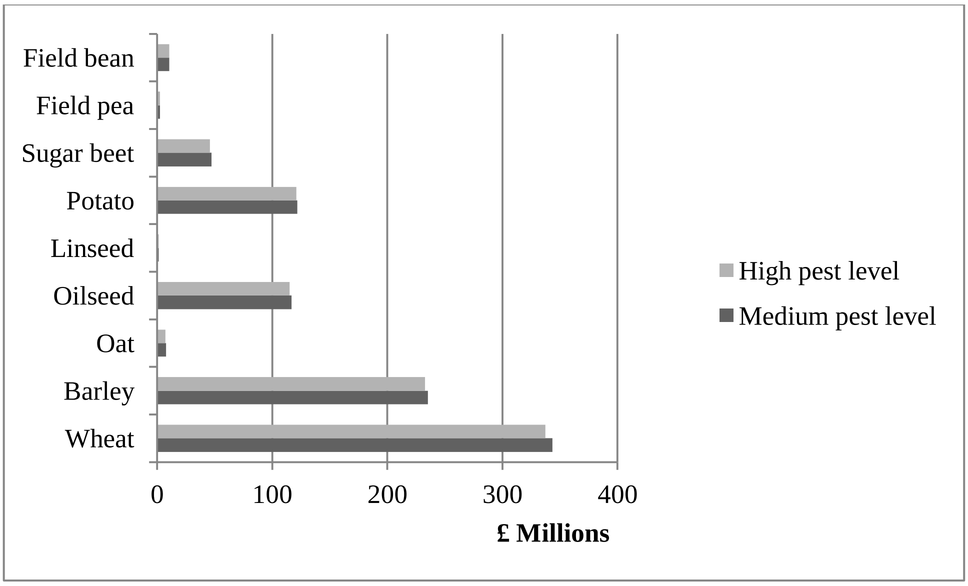

Figure 1. Estimated economic values of pest control service by arthropod natural enemies for UK wheat, barley, oat, oilseed rape, linseed, potato, sugar beet, field pea and field bean produced in 2013/14.

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Nitrogen fertilization alters host selection of pollen beetle parasitoids

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Abstract: Oilseed rape (*Brassica napus* L.) is a major agricultural crop that is endangered by pollen beetles (*Brassicogethes aeneus* Fab. syn. *Meligethes aeneus* Fab.). Due to the occurrence of resistance to commonly used synthetic insecticides, application of parasitoids to control *B. aeneus* herbivory is a promising alternative. Plants can attract parasitoids via the emission of volatile organic compounds (VOCs), the production of which is potentially dependent on the plant's physiological status which to a significant extent is driven by nitrogen availability. The purpose of the current research was to find the optimal N quantity to favor parasitation rate of pollen beetles in a controlled environment.

Oilseed rape plants were fertilized with four different N levels: 0, 80, 100, 160 kg N/ha. Out of 957 *B. aeneus* larvae collected in total, 90.7% were uninfested by parasitoids. In the remaining larvae, five parasitoid species were found to be responsible. Of parasitised larvae, 76.4% were infected once and the rest had two or more parasitoids. Although the number of pollen beetle larvae was much higher in N160, parasitation rate was greatest in N80. This may be related to N-dependent production of VOCs, which helps insects in host detection. These results suggest that the ecological control of pollen beetle herbivory is most promising under moderately high N addition rates (treatments 80 and 100) compared with non-fertilized or strongly fertilized treatments.

Key words: nitrogen fertilization, *Brassica napus*, *Brassicogethes aeneus*, *Meligethes aeneus*, pests, parasitoids, biocontrol

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Variation in abundance of pollen beetle, *Meligethes aeneus*, and its parasitoid, *Tersilochus heterocerus*, in oilseed rape in relation to proximity to woodlands, grasslands and other oilseed rape fields

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Abstract: The pollen beetle, *Meligethes aeneus*, is one of the most important pests of oilseed rape (OSR). It is known to be strongly influenced by the proximity of woodlands and grassland. Moreover, studies on the attractiveness of other species in the Brassicaceae suggested that trap crops near woodlands could help to contain their spread. Another option would be to preserve its main parasitoid, *Tersilochus heterocerus*, which can induce very high parasitism rates. Here we investigated jointly the impact of proximity to grassland and woodlands on the abundance of adults of both species. We first show there is no pollen beetle gradient within fields but a gradient up to 6,000 m even when intermediary OSR crops are present, suggesting trap crops would not efficiently halt pollen beetle. Multivariate regression confirms the strong positive impact on pollen beetle abundance of woodland density within 200 m and of grassland areas within 400 m. Only the presence of grassland significantly impacts the presence of parasitoids. This suggests that pollen beetle management could be improved by adequate temporal management of grasslands. For example, farmers could support grassland production of nectar-producing flowers during parasitoids' spring flight but mow them before the summer flight of the pollen beetle.

Key words: abundances, biocontrol, field scale, landscape, parasitoids

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Do different field bordering elements affect cabbage seed weevil damage and its parasitism rate differently in winter oilseed rape?

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Abstract: The cabbage seed weevil, *Ceutorhynchus obstrictus*, is an important oilseed rape crop pest in Europe. Its abundance is usually managed by synthetic insecticides that can be harmful to neutral and beneficial organisms, including parasitoids, occurring in the agricultural fields. Parasitoids can play an important role in the control of the population size of seed weevils. This experiment was conducted to see if and how different field bordering element types affect cabbage seed weevil infestation and parasitism rate in conventionally grown winter oilseed rape crops. The percentage of damaged pods was low (between 8.5% and 10.9%), but even with such low pest abundance the parasitism rate was sufficient for efficient biocontrol; varying between 55.5% and 68%.

Key words: *Ceutorhynchus obstrictus*, field margins, conservation biological control

Introduction

The cabbage seed weevil *Ceutorhynchus obstrictus* Marsham (Coleoptera: Curculionidae) is a significant brassicaceous oilseed crop pest in Europe and North America (Dosdall *et al*., 2001; Alford *et al*., 2003). Adult weevils feed on oilseed rape plants on two separate occasions during the season, but the main yield loss is caused by larval feeding inside the pods on developing seeds (Doucette, 1947; Free *et al*., 1983; Buntin, 1999). In the spring, emerged adults feed on buds and flowers of early flowering brassicaceous plants (Doucette, 1947) and invade winter oilseed rape during the bud and/or early flowering stage (Williams & Free, 1978), where they continue feeding, then mate and females lay their eggs, one or two at a time inside pods that are at least 2 mm in diameter (Doucette, 1947; Dmoch, 1965).

In Europe parasitoids play an important role in the population regulation of the cabbage seed weevil (Williams, 2003; Veromann *et al*., 2011; Kovács *et al*., 2013), however in most oilseed rape growing areas, control measures currently rely on synthetic pyrethroid insecticides applied as foliar sprays (Williams, 2003), which are effective but at the same time create a harmful environment for beneficial organisms. The over use of pesticides threatens the efficacy of biological control by killing natural enemies of pests. When natural regulatory processes are reduced, insecticide applications have to be increased in order to keep pests under control (Pickett *et al*., 1995), which might lessen the economic competitiveness of the crop. Additionally, the resistance of *C. obstrictus* to tau-fluvalinate, etofenproxand and lambda-cyhalothrin recorded in Germany (Heimbach & Müller, 2013) shows the importance of avoiding unjustified pesticide use to lessen the likelihood of pesticide resistance development. Hence, alternative management strategies should be favoured. Creating and maintaining alternative habitats for beneficial insects has to be a key task in pest management.

Non-cultivated field margins serve as hibernation sites and offer food sources for adult weevils, supporting their survival, especially if the vegetation is botanically related to the main crop (Altieri, 1999). On the other hand, these sites also provide regulating ecosystem services by maintaining a habitat for the natural enemies of many pests via providing a modulate microclimate (Rahim *et al*., 1991), overwintering habitat, food resources (Jervis *et al*., 1993), source of alternative prey, all of which results in higher densities of predators and parasitoids (Lys *et al*., 1994; Sutherland *et al*., 2001).

The aim of this study was to measure and compare cabbage seed weevil infestation and parasitism rate in oilseed rape fields bordered by different non-crop habitat types.

Material and methods

Study area

The study was carried out on commercial winter oilseed rape crops in 2013, Tartu County, Estonia. Four different commonly occurring landscape element types were selected: herbaceous areal, woody areal, herbaceous linear and woody linear. The dimensions of areal elements were at least 60 x 60 meters while the width of linear elements did not exceed 12.5 meters and the length was at least 150 meters. At the sampling point the study crop was directly bordered by one landscape element and the minimum distance to a different element was at least 200 meters. There were 54 sampling points; 19 on winter oilseed rape crops bordered by herbaceous areal elements, 11 by woody areal, 15 by herbaceous linear and 9 by woody linear elements.

Sampling method

The cabbage seed weevil damage and parasitism rate was assessed at the ripening stage of oilseed rape (BBCH 83-85) (Lancashire *et al*., 1991). Pod samples were collected from two different distances per sampling point, 2 and 20 meters from the field margin. At each distance 10 pods (5 pods from the main raceme and 5 pods from the third raceme) from 5 randomly selected plants were collected, 100 pods per sampling point. Collected pods were incubated for four weeks in emergence traps (described in detail in Dosdall *et al*., 2006; Dosdall & Kott, 2006; Veromann *et al*., 2011; Kovács *et al*., 2013) after which time the emerged larvae and parasitoids were counted. Pods were examined and dissected and all exit-holes and weevil or parasitoid remains were noted. The rate of damaged pods and parasitism rate was calculated per plant.

Statistical analyses

The effect of field bordering elements on the damage and parasitism rate were analysed using a generalized linear model with normal distribution and identity-link function. The differences between treatments were studied using the same analysis but with Bonferroni correction. Statistical analyses were carried out using STATISTICA 12 (Statsoft Inc. USA, 2014).

Results and discussion

Generally, the damage rate caused by seed weevils was low in all studied winter oilseed rape fields and approximated 10%. In our study, the bordering landscape element type of the oilseed rape crop had no effect on the damage rate caused by *C. obstrictus* ($\chi^2 = 2.40$, df = 3, $p = 0.49$). The damage rate was similar on all crops regardless their bordering landscape elements (Figure 1) probably due to the low average damage rate (9.9% ± 0.6%). However, despite the low damage rate, parasitism rate was high in all studied fields and exceeded 50%, although the crop bordering element type had no effect on the parasitism rate ($\chi^2 = 3.25$, df = 3, p = 0.35). The highest parasitism rate was recorded from crops bordered with herbaceous linear elements (68% ± 5.3%) and lowest on locations where oilseed rape was bordered with woody elements: 55.7% (± 7.4%) of *C. obstrictus* was parasitized next to woody linear elements and 55.5% (± 5.9%) next to woody areal elements (Figure 1). Although not statistically significantly different, a somewhat lower parasitism rate in oilseed rape crops adjacent to woody landscape elements might indicate difficulties in the host finding of parasitoids.

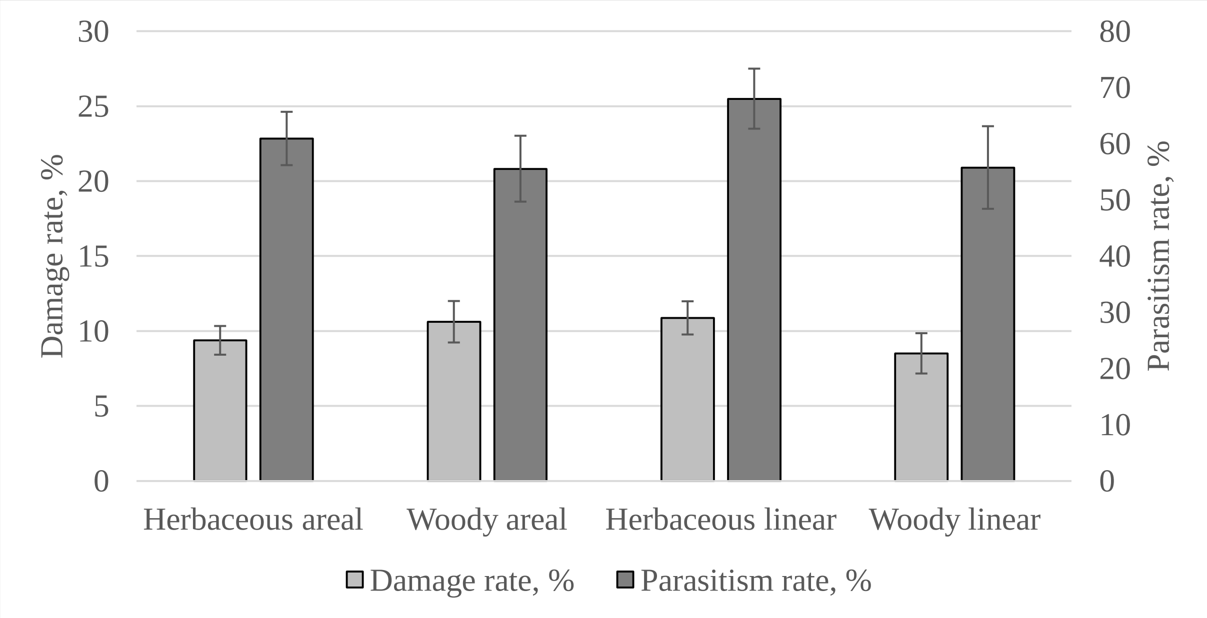

Figure 1. The mean (± SE) percentage of damaged winter oilseed rape pods caused by *Ceutorhynchus obstrictus* and its larval parasitism rates on crops adjacent to different landscape elements in Tartu County, Estonia, 2013.

Like other parasitoids, species attacking *C. obstrictus* use volatile cues emitted by host plants (Turlings *et al.*, 1991; McCall *et al.*, 1993; De Moraes & Mescher, 1998; Dicke, 1999); parasitoids of brassicaceous pests may use in addition isiothiocyanates, specific volatile organic compounds emitted by plants of this family, to locate and recognize host plants (Bartlet *et al.*, 1993; Alford *et al.*, 2003; Schiestl, 2010; Williams & Cook, 2010). Both oilseed rape pests and their parasitoids fly upwind towards the scent of host plants (Williams *et al.*, 2007). As the dispersal of volatile compounds is directly linked to physical barriers, woody elements (forests and hedgerows) suppress wind and therefore decrease the dispersal of odours indirectly affecting the host finding success of parasitoids. The odour plumes and their dispersal is known to differ in forests and open landscapes (Murlis *et al.*, 2000) and likely affect the dispersal of both pests and parasitoids. As pests only need to locate the crop they are in a better position compared to parasitoids, which first need to locate the crop and then the host within that habitat (Vinson, 1998) which might explain the equal damage rate between different crop bordering elements. Despite the lack of significant differences among parasitism rate, the average parasitism rate per sampling point type varied up to 5%, which suggests that parasitoids might be more affected by landscape composition than pests. However, the parasitism rate in all studied fields surpassed the level of effective pest control (32% according to Hawkins & Cornell, 1994) and we can conclude that in Estonia they can effectively control the population size of seed weevils.

In future studies it would be important to determine if and how much different field margins affect the damage rate and parasitism rate of this pest by comparing fields bordered by non-crop habitats with fields bordered directly by another crop field.

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Entomology Session 4 –
Breeding for resistance to insect pests for IPM strategies in OSR

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Screening different varieties of oilseed rape for sources of resistance against insects

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Abstract: Because of its long cultural cycle, oilseed rape is a crop particularly susceptible to multiple insect attacks. So far these attacks have mainly been controlled through the use of pesticides but such control methods are getting less efficient due to the development of resistance in several insect pest species, not to mention the many environmental and health problems they can pose. Among possible options for pest control, plant resistance is a good alternative but its feasibility in oilseed rape still needs to be explored for many of the problematic insects of this crop.

Damage to a crop by insects can be considered as resulting from (i) its attractiveness to colonizing adults, (ii) its ability to stimulate oviposition and/or feeding and (iii) its ability to ensure larval development. In the present study, we explore variability in these plant traits using several genotypes of oilseed rape for three pest insects attacking different plant parts, at different periods of the growing season: the cabbage stem flea beetle, *Psylliodes chrysocephala,* the cabbage root fly, *Delia radicum* and the pollen beetle, *Meligethes aeneus*. We explore this variability both in the field and in the lab and propose key traits that could be targeted for each of these pests in oilseed rape resistance breeding.

Potential for oilseed rape resistance in pollen beetle control

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Abstract: The pollen beetle (*Meligethes aeneus* F.) is one of the most damaging pests of oilseed rape (*Brassica napus* L.). A number of strategies are currently developed to combat this insect species, including synthetic and botanical insecticides, trap cropping and biological control by the pest's natural enemies. One strategy, plant resistance, although widely used in the management of many crop species, has not yet been developed against the pollen beetle despite a few notable attempts. Here we summarize the work of our laboratory on (i) the drawbacks to applying this strategy and how to circumvent them, and (ii) how the natural resistance present in oilseed rape may be used to limit pollen beetle damage in a theoretically effective and sustainable manner.

Key words: Chemical ecology, oilseed rape, phenotyping, plant resistance, pollen beetle

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Testing genotype susceptibility to insect pests: an example from the oilseed rape – pollen beetle interaction

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Abstract: Pollen beetle (*Meligethes aeneus*) is one of the main insect pests affecting oilseed rape crops. Efficiency of insecticide use to control this pest is decreasing due to its adaptation to phytosanitary products such as pyrethroids. In this context, alternatives to this kind of control need to be found. Breeding oilseed rape for resistance to insect attacks could be an interesting way to deal with this issue. Actually, the primary benefit of this approach is the ease of its use by farmers. However, it remains complicated to breed plants for insect resistance, especially using field tests on large genotype collections. Our knowledge of the chemical ecology of interactions between oilseed rape and pollen beetles could allow us to find biochemical markers of this resistance. In this way an indirect breeding approach based on makers of resistance could be adopted rather than direct confrontation of plants to insects. Laboratory tests have already shown that variation in resistance between oilseed genotypes could be explained by the biochemistry of bud tissues. These observations now need to be validated under field conditions. To test this, we conducted a multi-site experiment in France to observe resistance of different genotypes to pollen beetles. Over three locations we phenotyped pollen beetle damage and sampled buds in the field to analyse their chemistry. Here we present the results of this experiment.

Key words: oilseed rape, pollen beetle, resistance, biochemistry, plant breeding

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Semi-field and laboratory methods to screen oilseed rape genotypes for resistance to pollen beetles (*Meligethes aeneus* F.)

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Abstract: Screening of oilseed rape genotypes for resistance against insect pests is biased by various methodological constraints. In the present study, two methods commonly used for phenotyping were modified to assess the host preference and feeding intensity of pollen beetles (PB) on six/eight introgression lines (*S. alba* x *B. napus*) and a standard cv. of oilseed rape (Fenja) under semi-field and laboratory conditions.

A multi-choice, semi-field experiment was conducted in April 2014 to study the attractiveness of plants and the feeding damage by overwintered PBs. In a randomized block design with 10 replicates per genotype, potted plants (all in growth stage BBCH 55/57 and stem length of 15-20 cm) were transferred to a cereal crop adjacent to crops of oilseed rape and exposed to natural infestation by PBs for three days. The number of PBs per plant was counted seven times within a period of three days by beating the inflorescences onto a tray. Finally, all buds > 2 mm in size were scored for feeding damage and oviposition holes.

The same set of genotypes was also tested in no-choice and dual-choice feeding experiments under controlled laboratory conditions. Single excised buds 2-3 mm in size were offered to one field-collected overwintered PB in small transparent plastic boxes (30 replicates per genotype). The amount of bud tissue consumed within 24 h was measured by the loss of biomass, based on the fresh weight of buds at the start and the end of the experiment.

The attractiveness of plants and the level of feeding damage differed significantly between genotypes and showed the same ranking in both semi-field trials and lab experiments. The number of PBs per inflorescence, the percentage of damaged buds and the bud biomass consumed, respectively, on three introgression lines were significantly lower than on the other genotypes, which were more attractive and consequently suffered from higher feeding damage. The different feeding intensities between genotypes could be confirmed by dual-choice bioassays.

Thus, these methods have shown potential for reliable screening of a larger number of genotypes for their attractiveness to and feeding damage by PBs in further studies.

Key words: insect resistance, oilseed rape, screening methods, plant breeding

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Screening of introgression lines for antixenotic and antibiotic mechanisms of resistance to cabbage seed weevil (*Ceutorhynchus obstrictus* Marsham)

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Abstract: The cabbage seed weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) is an important pest of brassicaceous oilseed crops in Europe and North America. Currently, application of chemical insecticides is the only commercially used strategy to control adult weevils and reduce subsequent yield losses associated with larval feeding on seeds. In previous studies, *Sinapis alba* (L.) has been found to be resistant to *C. obstrictus*, although the mechanisms of resistance still remain unknown. In a two-year study, we determined the relative susceptibility of different introgression lines (*S. alba x B. napus*) to weevil attack under controlled conditions and in semi-field trials. Specifically, we assessed the oviposition preferences of adult females and different parameters of larval performance. Our results indicate that antixenosis and antibiosis are expressed by individual introgression lines, with stronger effects of antibiotic mechanisms of resistance observed in both years. In addition, the resistance of *S. alba* to *C. obstrictus* was confirmed in no-choice tests as well as in semi-field trials.

Key words: mechanisms of resistance, larval performance, *Ceutorhynchus obstrictus*

Screening of *Brassica napus*, *Sinapis alba* and introgression lines for antixenotic resistance to oviposition by cabbage root fly (*Delia radicum* L.)

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Abstract: The cabbage root fly (*Delia radicum* L.) is an important insect pest in European oilseed rape (*Brassica napus* L.) production. Host plant choice made by gravid females is a prerequisite for the successful development of larvae that feed on the root tissue of young plants. Breeding for host plant resistance to *D. radicum* is a promising approach for integrated pest management. The brassicaceous species *Sinapis alba* L. has been reported to show high levels of resistance to *D. radicum* larvae. Multi-choice oviposition experiments were conducted under greenhouse conditions to assess two susceptible *B. napus* cultivars, two resistant *S. alba* cultivars and six introgression lines (*S. alba* x *B. napus*) for their attractiveness to oviposition by adult *D. radicum* females.

We found that the *S. alba* cultivars were as attractive for oviposition as the *B. napus* reference, indicating no antixenotic resistance of *S. alba* to females of *D. radicum*. Moreover, host plant choice by the cabbage root fly was highly mediated by the stem base diameter of plants, as egg numbers significantly increased with increasing stem base diameters of plants.

As a consequence, mechanisms of antixenotic resistance are not responsible for the reduced susceptibility of *S. alba* to *D. radicum* and antibiosis seems to display the stronger mechanism of resistance to *D. radicum* attack.

Key words: insect resistance, plant breeding, oilseed rape, *Delia radicum*, oviposition

Acknowledgements

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Plant Pathology Session 1 – Resistance to blackleg

Assessment of the efficiency of major resistance genes against blackleg of oilseed rape in Germany

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Abstract: Blackleg disease, caused by *Leptosphaeria maculans* is one of the most important fungal diseases in oilseed rape (OSR) production word-wide. 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 are capable of evolving 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. The aim of this study was to determine the efficiency of major resistance genes to *L. maculans* in different oilseed rape growing regions in Germany by identifying the frequency of virulent isolates and determining the race spectra of *L. maculans*. We cultivated two oilseed rape cultivars in fields throughout Germany from 2011 to 2014. 'NK Bravour' harboring for the most part no known major genes against *L. maculans* (serving as trap crop) and 'Exocet' harbouring the major gene Rlm7 to observe resistance breakage in the field. In autumn and spring we collected true leaves with typical Phoma lesions to gain isolates of *L. maculans*. Single pycnidia isolates were tested with an oilseed rape differential set through cotyledon inoculation for their virulence to different major genes. The differential set consisted of 10 oilseed rape genotypes harboring the major genes *Rlm*1, *Rlm*2, *Rlm*3, *Rlm*4, *Rlm*7, *Rlm*9 and *LepR*1, *LepR*2 and *LepR*3. Thereby, the frequency of virulent isolates in a region was determined. Isolates showing the same virulence complement were grouped to the same race. The frequency of isolates being virulent to *Rlm*1, *Rlm*2, *Rlm*3, *Rlm*4 and *Rlm*9, respectively, was above 85%. Conversely, the frequency of virulent isolates to *Rlm*7 was very low (< 5%). Interestingly, the frequencies of isolates being virulent to the major genes *LepR*2 and *LepR*3 showed a considerable variability between different regions, ranging from 35% to 100%. There was no isolate showing virulence to *LepR*1. Most isolates belonged to two races with a high virulence complexity. Only *Rlm*7 and *LepR*1 are still mediating resistance in oilseed rape to German *L. maculans* populations.

Key words: Blackleg, major resistance genes, efficacy, oilseed rape, German growing regions

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Resistance to stem canker (*Leptosphaeria* spp.) in interspecific *Brassica* hybrids and rapeseed (*Brassica napus* L.) cultivars

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Abstract: Stem canker of brassicas (blackleg), caused by the fungal complex *Leptosphaeria maculans-L. biglobosa* is a damaging disease of oilseed rape (*Brassica napus*) worldwide. Nowadays, the incorporation of *L. maculans* resistance into *Brassica* lines with desirable agronomic and quality traits is a major concern for breeding programs. Every year new population and hybrid varieties are introduced to the market. The aim of this study was to identify the sources of genetic resistance to stem canker in *Brassica* hybrids obtained from the crossings between two high yielding cultivars of *B. napus* and *B. carinata*, *B. fruticulosa*, *B. rapa* ssp. *chinensis* and *B. rapa* ssp. *pekinensis* as well as to check the resistance and performance of winter oilseed rape cultivars under field conditions in the western region of Poland. The experiment was conducted in field conditions using the F_2 generation of these interspecific hybrids. Moreover, 44 cultivars of winter oilseed rape, officially registered in Poland by the Research Centre for Cultivar Testing (COBORU) and two candidate cultivars with *Rlm7* resistance gene were tested. Screening of plant susceptibility/resistance was done in 2015. The field experiment was done in a randomized complete block design with two replicates in two locations in the Wielkopolska region (Greater Poland), situated 80 km apart. Disease incidence was assessed in autumn, two months after sowing, on 50 plants per replicate, according to a 0-4 scale of disease severity. Furthermore, the determination of *Leptosphaeria* species was studied using Loop-mediated DNA Amplification (LAMP) method. The genotypes differed with their reaction to the pathogen. Forms with increased resistance to blackleg especially among *B. napus* × *B. carinata* and *B. napus* × *B. fruticulosa* hybrids have been found. In both locations, the cultivars bearing the *Rlm7* resistance gene showed significantly less symptoms of stem canker, as compared to cultivars with no *Rlm7*. The isolates originating from cultivars without the *Rlm7* resistance gene were mainly identified as *L. maculans*, whereas the isolates obtained from cultivars harbouring *Rlm7* resistance genes were scarce and belonged mostly to *L. biglobosa*.

Acknowledgements

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Collagen and keratin hydrolysates induce resistance against *Leptosphaeria maculans* in oilseed rape

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Abstract: Induced resistance to pathogens using various synthetic and natural compounds is suggested as an alternative to plant treatment with pesticides. Among these preparations biodegradable ones are especially of great importance. To be economically attractive, the source material of resistance-inducing compounds has to be low-cost and available in sufficient quantity. On the grounds of these requirements we were searching for resistance inducers in animal waste proteins originating from the food and leather industry. As this source material represents also troublesome waste, their utilization for the development of products with added value is highly desirable.

Our work was focused on protein hydrolysates prepared from the food by-product collagen, leather and fur, and feathers. The composition of the hydrolysates prepared by acidic and alkaline hydrolysis was analysed and their potential for plant defence system activation was investigated in oilseed rape (*Brassica napus*). Then, efficient hydrolysates were fractionated and biological activities of the fractions were tested against *Leptosphaeria maculans* both *in vitro* and *in vivo* in cotyledon tests. The application of the hydrolysates induced the expression of defence genes implicated in signalling pathways regulated by salicylic acid and ethylene. The results indicate that these animal proteins can serve as a valuable source of compounds utilizable in protection of oilseed rape.

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Plant Pathology Session 2 – Blackleg distribution, severity and chemical control

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Identification of new virulent races of *Leptosphaeria maculans* populations on oilseed rape in the UK

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Abstract: Phoma stem canker, caused by the fungal pathogen *Leptosphaeria maculans*, is a damaging disease on oilseed rape in the UK and can cause yield losses up to 50% if the disease is not controlled (Fitt *et al*., 2011). Currently, this disease causes UK annual yield losses > £ 100 M despite use of fungicides (http://www.cropmonitor.co.uk). With recent loss of the most effective fungicides through EU legislation, potential yield losses will increase (Mahmuti *et al*., 2009). Use of host resistance to control this disease is becoming ever more important. However, new sources of resistance are often rendered ineffective due to pathogen population changes from avirulent to virulent. There is a need to monitor emergence of new virulent races of *L. maculans* and prevent them from spreading into new regions and to investigate molecular mechanisms of mutation from avirulent to virulent in *L. maculans* populations. Phoma leaf spot assessment was done on twelve different oilseed rape cultivars with different resistance (*R*) genes with/without background quantitative resistance (Drakkar, DK Cabernet, Es-Astrid, LSF1238, LSF1241, Adriana, DK Extrovert, DK Exalte, Incentive, Harper, Amalie and Mentor) at six different sites (Woodhall Farm, Hertfordshire; Morley, Norfolk; Rothwell, Lincolnshire; Impington, Cambridgeshire; Trumpington, Cambridgeshire; West Farm Barns, Oxfordshire) in the UK (2015/16 cropping season). Cultivars with no *R* gene against *L. maculans* developed severe phoma leaf spotting compared to cultivars with *R* genes and background quantitative resistance. Cultivars with *Rlm7* gene had less phoma leaf spotting compared to cultivars with *Rlm1*or *Rlm4* resistance genes. Leaves with phoma leaf spots were collected from cultivar Drakkar (no *R* gene) from all the six sites and *L. maculans* isolates were obtained from the leaf lesions. Single pycnidial isolates (243) were obtained from leaf lesions and pathogen identification was done by morphology on PDA and will be confirmed by species-specific PCR. Changes in the frequencies of avirulent *AvrLm1*, *AvrLm4* and *AvrLm7* alleles in *L. maculans* populations at different sites in the UK are being investigated by inoculation of conidial suspensions on the cotyledons of a differential set of cultivars (Balesdent *et al*., 2005). The molecular events leading to virulence against *R* genes that are currently used (*Rlm1*, *Rlm4* and *Rlm7*) will be analysed by exploiting the *L. maculans* genome sequence (Rouxel *et al*., 2011) and *Brassica napus* genome sequence (Chalhoub *et al*., 2014) data.

The release of ascospores in the air was monitored by using Burkard spore samplers at four different sites (Bayfordbury, Hertfordshire; Langton Green Eye, Suffolk; Rothwell, Lincolnshire; Impington, Cambridgeshire) in the UK (2015/16 season) and the frequencies of *AvrLm1* and *AvrLm6* in the *L. maculans* ascospore populations will be identified by qPCR. Weather conditions such as rainfall, wind speed and temperature influence the maturation of pseudothecia and release of ascospores (Huang *et al*., 2005). The temperatures and rainfall were recorded daily at all the Burkard spore sampler sites by weather stations located near the sites. The minimum and maximum temperatures each day (average temperature was calculated) and daily rainfall (mm) were noted in the weather stations from September to February (2015/16 season). The pattern of major ascospore release differed between sites. The

first major ascospore release was observed in November at Bayfordbury and Eye sites. At Impington the first major ascospore release was in October and at Rothwell it was observed during September/October.

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Temperature sensitivity of *Brassica napus* resistance against *Leptosphaeria maculans*

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Abstract: Each year losses in UK oilseed rape production due to phoma stem canker cost *c.* £ 80 million. In an effort to control this fungal disease, farmers grow resistant cultivars. Cultivar resistance may be described as either quantitative or qualitative in nature. Quantitative resistance is generally controlled by several genes. In contrast, qualitative resistance is controlled by single, dominant major resistance (*R*) genes, such as *LepR3*, which protect against specific pathogen races. Qualitative resistance has commonly been found to rapidly become ineffective because single *R* genes exert selection on the pathogen population. There is a need to study temperature-sensitivity of oilseed rape *R* genes against *Leptosphaeria maculans*. It has been suggested that temperature resilience and durability of resistance may be linked. Thus, it is hypothesised that 'durable *R* genes are relatively insensitive to temperature increases. There is a need to study the effect of temperature on defence responses of cultivars possessing different *R* genes.

Five near-isogenic oilseed rape lines, differing only in presence of four specific *R* genes, will be challenged with *L. maculans* isolates possessing corresponding *Avr* genes at high (above 25 °C) and low temperatures (below 20 °C). RNA will be extracted from infected leaf tissue and qPCR will be used to compare the expression of genes associated with the defence response.

As a first step a preliminary experiment was done to determine the most suitable method for inoculation with *L. maculans* conidial suspensions. Four techniques were assessed and cotyledons of two-week-old seedlings of a susceptible line were inoculated by each of these methods. Subsequent symptoms were assessed at various time points post inoculation. Leaf lesion phenotypes were assessed in several ways, including image analysis of inoculated leaves and staining with trypan blue. All four methods produced symptoms of *L. maculans* colonisation. These symptoms, however, varied greatly in severity and time taken to develop. A second preliminary assessment will investigate the effects of two inoculation methods on the wound response. These assessments will determine the most suitable inoculation method in terms of uniform gene expression and minimal wound effects which will then be used in later experiments.

An antibody was raised against *LepR3* and will be characterised in terms of recognition specificity and subcellular localisation of the target protein. This will be useful for future biochemical and molecular work.

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Country-wide and temporal distribution of pathogens associated with phoma stem canker in the Czech Republic

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Abstract: In the Czech Republic, the increase in area sown with oilseed rape during the last two decades has been contributing to the increased appearance of a worldwide economically important disease of oilseed rape – phoma stem canker. Phoma stem canker is caused by two closely related fungal pathogens, *Leptosphaeria maculans* and *L. biglobosa*. The objective of this study was to assess the countrywide distribution of these two causal agents of the disease and their occurrence in oilseed rape tissues. In the growing seasons from 2007 to 2011, 1454 leaf samples with spots were visually identified based on symptoms and then analysed using species-specific PCR. Out of these, 39 and 15% were detected as *L. maculans*- and *L. biglobosa*-infected, respectively, in case of single species-infected samples, while 26% corresponded to the co-infection by both species. DNA of either one or both of *L. maculans* and *L. biglobosa* was not detected in 20% of the leaf spot samples. Furthermore, some isolates, that were collected from selected leaf spots and maintained in pure cultures, were identified based on pigment production during culturing on solid and in liquid media and PCR assay. In this case, the co-infection by *L. maculans* and *L. biglobosa* in a single leaf spot appeared as well. In years 2007-2012, 708 bases of oilseed rape plants divided into upper stem, lower stem, root collar and root parts were analysed using symptom identification and PCR. The proportion of plants in which *L. biglobosa* DNA was amplified was greater than that of plants with *L. maculans* DNA and 40% of tested plants were found to be co-infected by both *L. maculans* and *L. biglobosa*. According to our results, it appears that *L. maculans* is the predominant species in autumn, while *L. biglobosa* is more successful species than *L. maculans* in colonization of oilseed rape tissues in later growth stages of a plant in conditions of the Czech Republic.

Key words: phoma stem canker, oilseed rape, *Leptosphaeria maculans, L. biglobosa,* symptoms, PCR

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Decreasing the risk of severe phoma stem canker caused by *Leptosphaeria biglobosa* on winter oilseed rape

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Abstract: Air sampler data from four sites in the UK indicated that the pattern of ascospore release was similar between the sites, whereby ascospores of *Leptosphaeria* spp. were released from September 2015 to February 2016. However, the timing of first major ascospore and number of ascospores released differed between the four sites. Field trial results for effects of fungicides on control of phoma leaf spot severity indicated that both prothioconazole and penthiopyrad + picoxystrobin reduced the severity of *L. maculans* and *L. biglobosa* phoma leaf spots on some cultivars. There were differences in the effectiveness of the two fungicides in control of phoma leaf spotting between the cultivars. Prothioconazole reduced the severity of *L. maculans* phoma leaf spots on two cultivars and *L. biglobosa* phoma leaf spots on three cultivars. Penthiopyrad + picoxystrobin reduced the severity of both *L. maculans* and *L. biglobosa* phoma leaf spots on three cultivars.

Key words: Phoma stem canker, *Leptosphaeria maculans*, ascospores, prothioconazole, penthiopyrad + picoxystrobin, phoma leaf spots, AHDB recommended lists

Introduction

Oilseed rape is the third most important arable crop in the UK. Phoma stem canker is a damaging disease of oilseed rape that causes £1000M yield loss worldwide per growing season (Stonard *et al*., 2010; Huang *et al*., 2011). This economically important disease is caused by two closely related fungal pathogens; *Leptosphaeria maculans* (Lm) and *Leptosphaeria biglobosa* (Lb) (Figure 1). Lm is thought to cause more damage to the crop by forming stem base cankers, whereas Lb has been generally associated with the less damaging upper stem lesions (Toscano-Underwood *et al*., 2003; Fitt *et al*., 2006). Therefore, more research has been done on Lm and very little work has been done on Lb.

Recent studies have suggested that Lb can cause both upper stem lesions and stem base cankers, leading to severe yield losses (Huang *et al*., 2014; Liu *et al*., 2014). Furthermore, Lb is less sensitive to some triazole fungicides than Lm (Huang *et al*., 2011) and no cultivar resistance has been bred against Lb (Fitt *et al*., 2006). This study aims to understand the importance of Lb in phoma stem canker epidemics in UK and to improve the disease control by targeting both Lm and Lb.

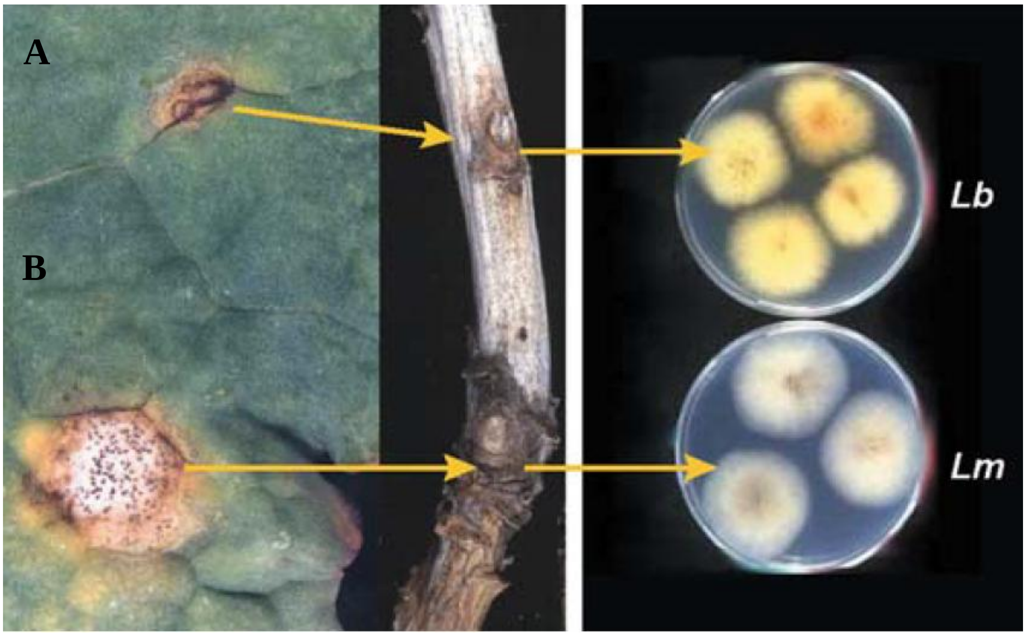

Figure 1. Symptoms of *L. maculans* and *L. biglobosa* on leaves, stems and potato dextrose agar (PDA). (A) *L. biglobosa* is generally associated with small dark lesions on the leaf surfaces, upper stem lesions and yellow pigmented fluffy colonies on PDA. (B) *L. maculans* is generally associated with large pale lesions on leaves, stem base cankers and pale white colonies on PDA (Figure adapted from Figure 1, Fitt *et al*., 2006).

Material and methods

Burkard air samplers were set up at four sites in the UK to monitor the timing and number of ascospores released from September 2015 to February 2016. The spore tapes of the air samples were cut into two halves; one half was used for spore counting and the other half was stored for DNA extraction and qPCR analysis to differentiate Lm and Lb in the *Leptosphaeria* populations. A winter oilseed rape field trial was set for the 2015/2016 growing season at ADAS Boxworth. Six cultivars with different levels of 'field' resistance to phoma stem canker were used in a randomized block design experiment with three replicates. The cultivars with their AHDB (Agriculture and Horticulture Development Board) recommended list 'field' resistance rating have been listed in Table 1 (AHDB, 2015). These cultivars were treated with two fungicides; Prothioconazole and Penthiopyrad + picoxystrobin. Severity of phoma leaf spots caused by Lm or Lb was assessed. Leaf samples were collected for obtaining Lm and Lb isolates.

Table 1. List of cultivars with (AHDB recommended list resistance rating) used in field experiment at ADAS Boxworth (Phoma stem canker resistance rating based on AHDB Recommended list; winter oilseed rape 2015/2016 – East/West Region). Resistance rating range = 0-9; 0 = Highly susceptible; 9 = Highly resistant (AHDB, 2015).

List of cultivars	**AHDB recommended list resistance rating for cultivars in 2015/2016**
DK-Cabernet	6
Fencer	8
Harper	8
Incentive	4
PR46W21	3
Quartz	9

Results and discussion

Data from air samplers showed that the timing of first major ascospore release and number of ascospores released differed between the four sites (Figure 2). The general patterns of ascospore release were similar among the four sites, with the release of ascospores starting from mid-September and finishing in February.

In the field experiment at Boxworth, there were differences between different cultivars in the severity of phoma leaf spots caused by Lm and Lb. There were differences between the two fungicides in control of phoma leaf spots; prothioconazole reduced the severity of Lm on two cultivars and severity of Lb on three cultivars, whereas penthiopyrad + picoxystrobin reduced the severity of both Lm and Lb on three cultivars.

The timing of ascospore release can be used to guide the timing of fungicide applications (Huang *et al.*, 2007). Studies on effects of fungicides on control of phoma stem canker in different cultivars can help in choosing effective fungicides in relation to the cultivars in the local areas. Further controlled environment experiments and fungicide sensitivity tests will be done to investigate the control of phoma stem canker epidemics, targeting both pathogens.

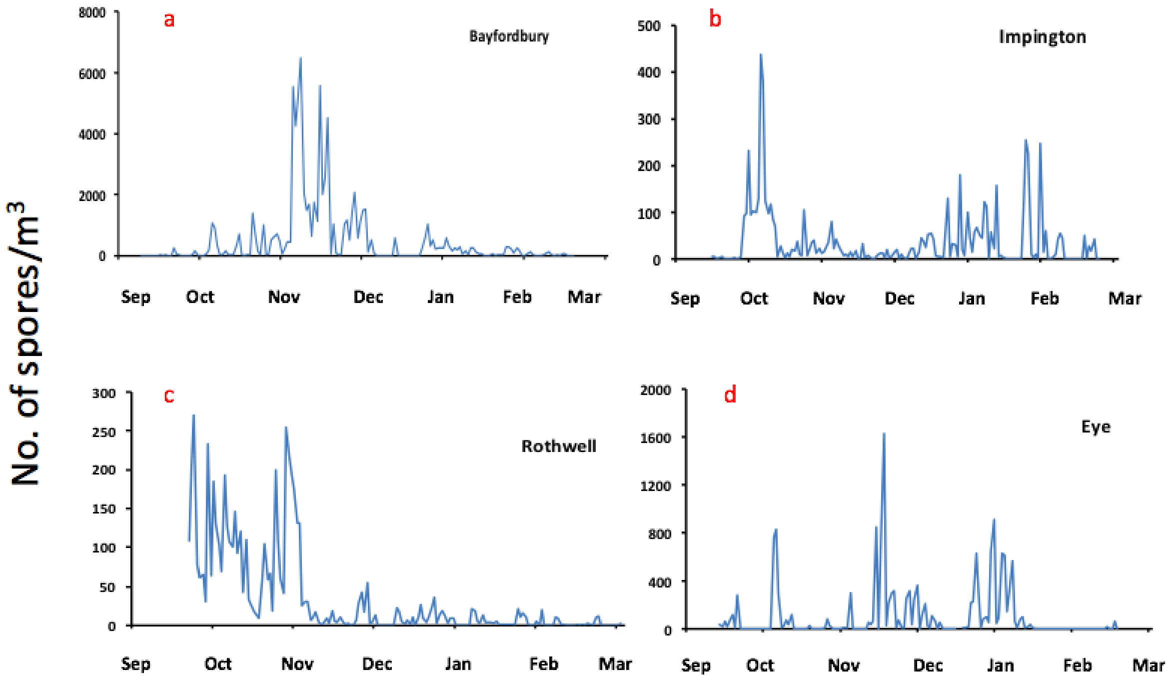

Figure 2. Burkard air sampler data for 2015/2016. Ascospore data collected for the four sites in the UK. Spores were counted on the spore tapes using a light microscope for September 2015/February 2016 at (a) Bayfordbury, Hertfordshire (b) Impington, Cambridgeshire (c) Rothwell, Lincolnshire (d) Eye, Suffolk.

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The effects of different plant growth regulators and fungicides on Phoma stem canker, growth parameters and the yield of winter oilseed rape

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Abstract: The purpose of the current study was an estimation of the influence of application of plant growth regulators (PGR) and fungicides on growth of oilseed rape (OSR), blackleg disease development, plant wintering and plant yield. A multifactorial field experiment with 4 replications was designed in split plots in Ahlum, Lower Saxony, Germany from 2012/2013 to 2014/2015. The plots consisted of 4 OSR cultivars (Elektra, Vitara, PR 46W20 and Genie) and subplot treatments were 5 different PGRs (Ampera, Carax, Folicur, Tilmor and a combination of Imbrex/Folicur) which were applied twice; in autumn (BBCH 14-18) and in spring (BBCH 30-55). Observations such as growth parameters like plants/m^2 and plant height, yield parameters like number of pods/plant, number of seeds/plant, thousand grain weight, seed yield and also evaluation of Phoma disease severity were taken using EPPO standard procedures. The data of the individual parameters were evaluated separately for each year of the field experiment. PGRs application effect on plant survival during winter was not observed for treatments. In contrast, the application of PGRs and fungicides had a substantial influence on the control of Phoma leaf symptoms in autumn. Hereby, Phoma leaf disease in autumn was not significantly correlated with OSR seed yield. In contrast, Phoma stem canker at growth stage 81-83 had a negative correlation with TGW and yield. The plant height at harvest stage varied significantly due to the different treatments. Among all tested yield factors, only number of plants/m^2 and pods/plant had a significant effect on OSR TGW and seed yield.

Key words: *Brassica napus, Phoma lingam*, yield parameters, disease severity

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Variability in fungicide sensitivity of *Leptosphaeria maculans* and *L. biglobosa*, the causal agents of blackleg disease in oilseed rape

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Abstract: Blackleg is a disease of world-wide importance on oilseed rape (*Brassica napus*), which can cause serious losses in different countries. The disease is caused by closely related pathogens *Leptosphaeria maculans*, causing stem-base canker and *L. biglobosa*, causing upper stem lesions late in the growing season. In 2015, in some regions of Germany relatively late disease symptoms were observed on the upper part of stems, causing the crown canker which was responsible for lodging of the plants and yield losses. The results of morphology and species-specific PCR assays revealed that *L. biglobosa* constituted 58% of all isolates obtained from the infected stems. In general, besides growing resistant oilseed rape cultivars, fungicide application showed a significant reduction in the percentage of blackleg disease incidence and severity but little is known about the differences of sensitivity of *Leptosphaeria* spp. isolates to different fungicides. In the present study, the effects of the most important groups of fungicides (QoI, SDHI, DMI and MBC) were examined on the germination of pycnidia and the inhibition of mycelial growth of 10 reference strains of *L. maculanse* and *L. biglobosa*. Fungicide sensitivity tests *in vitro* were conducted using fungicide amended agar plates at 0.0, 0.001, 0.01, 0.1, 1.0, 10.0 and 100.0 µg a.s./ml concentrations. The results show that the two pathogens did not differ significantly in their growth rates under *in vitro* conditions. Lower concentrations of all fungicides (0.001, 0.01, 0.1 and 1.0 µg a.s./ml) have no or a low effect on conidial germination and mycelial growth inhibition in either species. In contrast, at higher concentrations significant differences in growth inhibition were observed between *L. maculans* and *L. biglobosa* isolates when treated with fungicides. *Leptosphaeria maculans* isolates were significantly more susceptible to all fungicide groups than *L. biglobosa*. Even at the highest concentrations (100 µg a.s./ml) none of the fungicides could achieve 50% control of *L. biglobosa* in either the conidial germination test or the mycelial growth inhibition assay.

Key words: *Phoma lingam*, *Brassica napus*, foliar fungicide, stem base canker, disease assessment

Plant Pathology Session 3 –
Non-chemical control of diseases

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The potential of *Trichoderma* strains for control of stem canker of brassicas (*Leptosphaeria* spp.)

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Abstract: Oilseed rape (*Brassica napus* L.) is one of the most expanding crops worldwide. Currently, European Union is producing the highest amount of oilseed rape seeds and oil, but it is followed by numerous other producers, such as China, Canada and Australia. The increase of oilseed rape production and its common use in crop rotations causes problems in crop protection against many pathogens, especially fungi. Many countries in Europe are currently facing problems with the reduced number of registered active substances present in fungicides. The obligatory system of integrated crop management requires decision support systems and a number of methods that avoid chemical compounds and promote other means of disease control. This included a number of useful agrotechnical strategies as well as compounds which enhance resistance and protect the plants with antagonistic microbes, harmless to humans and animals. We have studied the potential of the genus *Trichoderma* to control stem canker of brassicas – one of the most damaging diseases of oilseed rape worldwide. The genus *Trichoderma* proved great potential for biocontrol in many crops and the first attempts to control rapeseed diseases were also very promising.

Our study demonstrated that *T. harzianum*, *T. hamatum* and *T. longibrachiatum* can help to control phytopathogenic fungi *Leptosphaeria maculans* and *L. biglobosa*. Several species of *Trichoderma* affected the growth of these pathogens and to some extent, they have also decreased disease severity in field experiments. On top of this, spraying with conidiospores in the autumn was helpful in reducing the incidence and severity of phoma leaf spots. It has also accelerated the degradation of plant stubble and the decomposition of pseudothecia. All *Trichoderma* isolates showed higher cellulolytic activity and enhanced resistance to flusilazole treatments, which coincided with upregulation of 14α-sterol demethylases and an AbcG5 transporter. The effects we observed justify the use of *Trichoderma* to enhance the resistance of oilseed rape against pathogens, which in turn may lead to a decrease in the use of pesticides.

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The influence of antagonistic fungi on the growth of *Sclerotinia sclerotiorum*

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Abstract: *Sclerotinia sclerotiorum* is a pathogen of many crops, including rapeseed. It causes diseases which have serious economic consequences. The pathogen can be controlled with fungicides. There are also studies on the use of antagonistic fungi to limit the development of *S. sclerotiorum*.

The study assessed the influence of antagonistic fungi on the growth of *Sclerotinia sclerotiorum* isolates. Five cultures of antagonistic fungi were used: *Trichoderma* sp., *T. viride*, *T. harzianum*, *Ulocladium atrum*, *Coniothyrium minitans* and four *Sclerotinia sclerotiorum* isolates. The isolates were inoculated on a potato dextrose agar (PDA) in Petri plates (9 mm) by placing rings (5 mm) of the mycelia of the pathogen and antagonist at a distance of 6 cm from each other. Isolates of the pathogens and antagonists inoculated individually on a PDA were used as control samples. The experiment was conducted at a temperature of 21 °C. During the following days of incubation the influence of the antagonists on the growth of *S. sclerotiorum* was assessed by measuring the radius of the fungal colonies and comparing it with the values observed in the control samples.

Apart from *C. minitans*, all the antagonistic isolates inhibited the growth of *S. sclerotiorum*. As far as the biculture of the pathogen with *T. viride* is concerned, the growth of both colonies continued until their mycelia contacted each other. *U. atrum* caused the formation of a noticeable inhibition zone of several millimetres, which remained until the end of the experiment. *T. harzianum* and *T.* sp. continued growing and successively covered the whole mycelium of the pathogen. Among the antagonistic fungi, the *Trichoderma* sp. isolate had the most inhibitory effect and covered the *S. sclerotiorum* mycelium most intensively.

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The influence of crop rotation and the time of application of fungicides on the occurrence of perpetrators diseases in winter oilseed rape

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Abstract: The agrotechnical method belongs to the methods used in the integrated crop protection of winter oilseed rape (OSR) against fungal diseases. Crop rotation remains one of its underestimated components, especially when it comes to keeping long enough intervals between growing crops of the same group. Some of the best forecrops for OSR are the different types of legumes. However, for the natural and economic reasons, OSR is usually grown after cereals. The higher the OSR share in crop rotation, the higher the risk of pests, including fungal diseases. During the growing seasons of 2012/2013-2014/2015, the experimental field at the Plant Protection Institute – National Research Institute, Sosnicowice Branch (south-western Poland) and the Field Experimental Station in Winna Gora (mid-western Poland) were the sites of controlled plot trials. Winter OSR Visby F1 was grown in three different crop rotation schemes: 3-4 years after OSR, after wheat and in monoculture. To protect the crop against *Sclerotinia sclerotiorum* and pod diseases, the researchers selected three fungicides, approved for use in Poland, based on active substances that belong to different chemical groups (prochloraz, azoxystrobin, dimoxystrobin+ boscalid) and applied them at three different timings, i.e.: at the beginning of flowering (BBCH 60-61), full flowering (BBCH 64-65) and end of flowering (BBCH 69-70). The trials were designed to assess the effect of crop rotation and fungicidal treatments used at the flowering stage on fungal diseases in OSR. At the stage of pod formation, researchers assessed the percentage of plants infected with *S. sclerotiorum*. At the same time, they analyzed the pod infection and the findings were presented as % of infected pods. The trial also assessed the seed yield and TGW. The findings underwent statistical analysis. Statistical calculations were performed using the Statistica 8.0 software and ANOVA analysis of variance. The results were compared using Duncan's test with significance level of $P < 0.05$. The results indicate that the trial location, crop rotation and treatment timing had an effect on occurrence of fungal diseases. OSR infection was highest in monoculture and second highest in crop rotation when OSR followed wheat. Infections increased with the increasing share of OSR. In crop rotations with higher OSR share, treatments applied at the stage of full bloom worked best against the different diseases, especially *S. sclerotiorum*. Seed yield was tied to the crop rotation scheme and the timing of fungicidal treatment. Due to the different growth rate of fungal diseases, the most effective timing for treatment applied at the flowering stage in mid-western Poland was the beginning to full blooming of OSR (BBCH 61-65), whereas for the south-western Poland, it was the from the full bloom until the end of blooming (BBCH 64-70).

Key words: winter oilseed rape, fungicide, disease, yield, crop rotation

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The effect of cultivation systems and crop rotation on the occurrence of weeds and diseases in oilseed rape

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Abstract: Recently the area of agricultural crops based on no-tillage cultivation systems has increased significantly in Poland. The simplified tillage system, used under good soil conditions with proper agricultural technologies enables maintenance of crop yield at a good stable level. Oilseed rape is an exception as it requires properly cultivated soils.

The aim of the long-term experiment set up in 2011 was to compare the effect of selected agricultural factors (cultivation system, crop rotation with a variable protection level) on the incidence of fungal diseases and weed infestation in winter oilseed rape. The tillage system was the first factor tested: I – reduced tillage; II – ploughing. The protection level was the second factor tested: b_1 – standard (current practice); b_2 – integrated (low pesticide input, including non-chemical methods). In standard practice (b_1) winter oilseed rape was sown in rows spaced at 24 cm, with chemical weed control and winter wheat as a stable forecrop. In the integrated system (b_2) band-row sowing was used (33 cm band sowing with a 50 cm inter-row). The inter-row was mechanically weeded with a hoe and herbicides were applied only for band spraying of oilseed rape rows; narrow-leaved lupine was used as a forecrop.

In both tillage systems the same protection measures were used against pests (TFI value). As far as the second factor is concerned, the level of protection varied and the total TFI value (the sum of herbicide and fungicide) was as follows: $b_1 = 1.97$ and $b_2 = 1.0$. The following broad-leaved weed species were predominant: *Centaurea cyanus*, *Geranium pusillum*, *Viola arvensis*, *Matricaria inodora*, *Anchusa arvensis* and grass weed species: *Elymus repens* and *spica-venti Apera*. In the simplified tillage system weed infestation was significantly higher than in the ploughing system.

The experiment revealed that oilseed rape samples had symptoms of infection with grey mould (*Botryotinia fuckeliana*), stem canker (*Leptosphaeria* spp.), stem rot (*Sclerotinia sclerotiorum*) and black spot (*Alternaria* spp.). The incidence of rape diseases mostly depended on the weather conditions in a particular season. During the first season there was no stem rot infection observed. The highest incidence of stem rot in oilseed rape was recorded in the second year. In the same year a significant percentage of oilseed rape plants was infected with stem canker, whereas oilseed rape siliques showed the symptoms of grey mould and black spot. Occasionally, the occurrence of fungal diseases depended on crop rotation and on the cultivation system. In the reduced tillage system there was a greater percentage of infected plants, especially with the pathogen causing stem rot.

The results showed that despite the reduced use of chemicals in the integrated system weed infestation and fungal infection decreased, whereas the yield of oilseed rape increased. There were more evident differences between protection practices in the reduced tillage system. In the ploughing system the yield of winter oilseed rape was significantly higher than in the reduced tillage system, regardless of the protection level and crop rotation.

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Integrated management strategies for controlling light leaf spot (*Pyrenopeziza brassicae*) in winter oilseed rape

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Abstract: Reports of light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, in commercial winter oilseed rape crops in Great Britain have been increasing in Scotland, England and Wales. In Scotland, 62% of crops were reported to have light leaf spot symptoms in spring 2015, and in England and Wales 85% of crops were affected. Yield losses caused by light leaf spot in England and Wales were estimated to be in the region of £ 140 million (€ 178 million) despite the majority of oilseed rape crops receiving at least 3 fungicide applications in a single season. Control of light leaf spot is predominately through the application of two spray fungicide programmes, with the first usually in November followed by another fungicide application pre-/at stem extension onwards (usually February/March) and using varieties with disease resistance. Growers in the UK can select varieties based on a range of characteristics described in the Recommended Lists® for cereals and oilseeds which are published annually by the Agricultural and Horticultural Development Board (AHDB). Independent information on efficacy and appropriate fungicide doses for disease control including light leaf spot for a range of fungicide modes of action are also available from the AHDB, however, despite the integration of strategies to control crop diseases being encouraged, there is relatively little information available on the benefits such strategies can offer growers. This paper examines data derived from experiments using combinations of varietal resistance and fungicide application timing for light leaf spot control in 2015 from Scotland and England. The benefits of using integrated control strategies for control of light leaf spot, as well as the potential impact for control of other diseases, will be discussed.

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SDHI resistance in French populations of *Sclerotinia sclerotiorum* and its management

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Abstract: *Sclerotinia* stem rot is responsible for the most severe attacks on oilseed rape in France, and therefore, is controlled by 1-2 sprays of fungicides. The use of SDHIs (succinate dehydrogenase inhibitors), mostly represented by boscalid, led to the selection of low to high resistant strains, as measured *in vitro*. This resistance evolved and spread in French populations of *Sclerotinia sclerotiorum*. It was systematically correlated in our sampling with one of the seven changes detected in the subunits B, C or D of the target enzyme of SDHIs using molecular tools. Although reduced field performance is infrequent, recommendations for growers are provided to deploy appropriate resistance management strategies.

Key words: *Sclerotinia sclerotiorum*, resistance, boscalid, SDHI

Pasmo: observations of pseudothecia of *Mycosphaerella linicola* on linseed stubble

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Abstract: Pasmo is a major disease affecting linseed. Infected linseed stubble were observed during the autumn, showing not only the presence of pycnidia of *Septoria linicola* but also more pseudothecia, indicating the existence of the teleomorph *Mycosphaerella linicola* in France, potentially responsible for primary contamination of winter linseed.

Key words: Linseed, pasmo, pseudothecia, *Mycosphaerella linicola*

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The comparison of different nutrition and growth stimulation programs on fluorescence of chlorophyll *a* and gas exchange efficiency in leaves of oilseed rape

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Abstract: Oilseed rape is the most widely cultivated crop species in the Brassicaceae family today and has now become the third leading source of vegetable oil and oil meal in the world. The acreage of cultivated oilseed rape plants is increasing yearly. In Poland, oilseed rape is the most important crop grown for industrial purposes, and there is potential for increasing the cultivation area of this crop. Nowadays, to obtain the highest possible yield, it is important to ensure growth conditions close to optimal. In the field, plants are often exposed to several stressful conditions therefore using growth stimulation programs and optimal nutrition to improve plant status has become quite common practice.

The aim of this work was to study the effect of different nutrition and growth stimulation programs on fluorescence of chlorophyll *a* and gas exchange efficiency in leaves of oilseed rape. Oilseed rape cv Saveo F1 (Syngenta Seeds) plants were cultivated in the 2015/2016 growing season at Ksiaz Wielkopolski in Poland. Biostimulant Dynamic Cresco 0.8 l/ha was applied as a single foliar spray. Two different 'optimal' nutrition programmes were also tested (Opti-1 and Opti-2). The following parameters of intensity of transpiration were measured using an infra-red gas analyser method (Photosynthesis System Licor 6400X): intensity of photosynthesis (Pn), stomatal conductance (gs), intercellular CO_2 concentration (Ci), transpiration rate (Tr), water use efficiencies (Pn/Tr), RuBP carboxylation limitation (Pn/Ci) and relative chlorophyll content (Chl). Chlorophyll *a* fluorescence was recorded with Handy Pea (HansaTech Inc.). The efficiency of excitation energy capture by open PS II reaction centers (Fv/Fm) and the quantum yield of electron transport at photosystem II were determined.

Application of Dynamic Cresco and the optimal nutrition program Opti-1 had positive effects on the most of the studied parameters and processes. Plants treated with the biostimulant showed more efficient gas exchange. The fluorescence of chlorophyll *a* also increased in response to the application with Dynamic Cresco. It suggests the biostimulant may play a protective role in oilseed rape plants, such as it was already observed during drought stress in spring 2016.

Plant Pathology Session 4 – Clubroot of oilseed rape

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Plasmodiophora brassicae Wor. on winter oilseed rape in the Czech Republic

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Abstract: Clubroot disease, caused by *Plasmodiophora brassicae* (Wor.), has been spreading on winter oilseed rape (*Brassica napus* L.) in the Czech Republic over the past five years. Research on *P. brassicae* in the Czech Republic is therefore important for the development of effective strategies to manage clubroot under Czech environmental conditions. Clubroot infestation and spread were monitored over five years and a map of infestation was created. Experiments with clubroot resistant cultivars of winter oilseed rape were carried out in the field and greenhouse. In the greenhouse, six clubroot resistant cultivars were grown in infested soil collected from various fields in the Czech Republic, and assessed for disease severity. The soil samples were also tested for the presence and amount of *P. brassicae* inoculum by conventional and quantitative PCR analysis. In the field experiment, seven clubroot resistant cultivars were grown and disease development was monitored monthly. Yields were measured at the end of the cropping season. Finally, a set of 17 *P. brassicae* field isolates from across the Czech Republic were assessed for pathotype designation on the differential hosts of Williams, Somé *et al.*, and the European Clubroot Differential set. Collectively, the information obtained on the effectiveness of host resistance and pathogenic diversity of *P. brassicae* populations from the Czech Republic may help to more effectively manage clubroot in this country.

Key words: *Brassica napus* L., clubroot disease, monitoring, field experiments, resistant cultivars, quantitative PCR, pathotypes

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Incidence of *Plasmodiophora brassicae* and the composition of its pathotypes in Poland

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Abstract: Oilseed rape is susceptible to a number of diseases that cause significant economic losses to farmers. Clubroot disease caused by the pathogen *Plasmodiophora brassicae* is a serious and still growing problem for oilseed rape growers on all continents and in many countries. The aim of this study was to determine the incidence and evaluate the pathotype composition of *P. brassicae* populations from Poland, according to three well known classification systems. Moreover, the pathotypes were designated based on two different thresholds: 1) Disease Index (ID) < 25%, as proposed by Somé et al (1996); 2) ID < 50% with the 95% confidence interval not exceeding 50%, as used by LeBoldus *et al*. (2012). There were considerable differences between the populations of *P. brassicae* using the various systems and different thresholds within each system.

Based on a threshold of ID < 25%, a total of five pathotypes were identified using the differentials of Williams (1966), including 44% of pathotypes classified as the pathotype 7. Meanwhile using ID < 50% seven pathotypes have been found, with 6 and 7 present in equal amounts (25% each). According to the European Clubroot Differential set (Buczacki *et al*., 1975) there were nine pathotypes each timein each case, with six identical common and three different identifications, depending on the threshold. Only the system described by Somé *et al.* (1996) classified the isolates to identical categories, regardless of the threshold. Molecular detection of *P. brassicae* using Real-time PCR showed very high incidence of this microorganism in numerous soils. The incidence of clubroot depended on soil pH and, intensity of oilseed rape cultivation as well as soil moisture.

Key words: *Brassica napus*, clubroot, European Clubroot Differential, soil test, quantitative PCR

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Plasmodiophora brassicae infection – usurping the host molecular regulatory networks for feeding site formation

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Abstract: Plants infected by *Plasmodiophora brassicae* are subjected to extensive change in developmental programs. During disease progression the pathogen needs to build an efficient feeding site, securing in this way successful production of resting spores. This developmental reprogramming is an outcome of interaction between the pathogen and infected host. During the disease progression we can observe change in growth regulator dynamics, altered patterns of cell proliferation and differentiation, increased cell expansion or eventual cell wall degradation as well as the redirection of nutrients towards the pathogen. Here we would like to present recent progress in molecular and anatomical studies describing developmental changes occurring during the infection. With the help of an integrative biology approach we were able to describe mechanisms leading to change in cell fate determination triggered by *P. brassicae* in the infected host plant. We also found the link between developmental reprogramming and the usurpation of the host nutrition distribution system by *P. brassicae*. This work summarises the current state of knowledge on *P. brassicae* driven developmental reprogramming of plants and suggests ways to study exact role of observed changes in the host.

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Proteomic approach to study cell wall changes occurring within host plant during clubroot infection

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Abstract: *Plasmodiophora brassicae* is the obligatory pathogenic protist causing clubroot disease in agriculturally important Brassica species. The disease has a detrimental effect on plants and leads to severe yield losses. The characteristic symptom of clubroot is the development of galls on the underground parts of the infected plants (Ludwig-Müller & Schuller, 2008). These structures resulting from hypertrophy and subsequent hyperplasy of cells (Malinowski *et al*., 2012) are responsible for plant wilting and eventual plant death. The expansive gall growth is caused by the formation of giant cells which constitute final reservoirs of resting spores of the pathogen (Mithen & Magrath, 1992; Schuller *et al*., 2014). Clearly, these changes observed during disease progression must be accompanied by massive cell wall modifications, which are typically regulated by cell wall remodelling enzymes e.g. cellulases, endotransglycosylases of xyloglucan and expansins. We believe that detailed characterisation of the cell wall remodelling process during *P. brassicae* infection will help to understand the mechanism used by the pathogen for invading plant cells and securing resting spore formation. Therefore, our research aims to identify the specific factors involved in the cell wall turnover process accompanying characteristic cellular responses observed during clubroot infection. In order to accomplish this task we will perform comparative proteomic studies by monitoring changes in cell wall protein profiles in galls (including giant cells) against appropriate non-infected controls. The experiments are based on the model plant *Arabidopsis thaliana* and involve the sequential cell wall protein isolation method (Feiz *et al*., 2006) combined with 1-D and 2-D protein electrophoresis, followed by the mass spectrometry-based identification of proteins with differential abundance between treatments. It is highly likely that not only are structural features of cell wallchanging, but also some cell wall proteins that may act as signalling molecules during this process. These may include arabinogalactan proteins (AGPs) - a heterogenous group of proteins composed of polypeptide, glycan and lipid parts (Seifert & Roberts, 2007). In order to characterise their involvement, we are planning to use a series of commercially available antibodies for *in situ* detection within developing giant cells. Ultimately, we plan to generate transgenic lines with modified activities of the identified and selected cell wall proteins and test the potential impact of such modifications on *P. brassicae* pathogenesis.

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Suppression of *Plasmodiophora brassicae*, an emerging pathogen of German oilseed rape crop, with soil amendments

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Abstract: Clubroot, caused by *Plasmodiophora brassicae*, is a major disease of cruciferous crops that is widely dispersed through the world. The oilseed rape production area in Germany is roughly 1.5 million hectares per year and oilseed rape remains an important crop in the rotation. Nowadays, clubroot disease is an increasing problem not only to oilseed rape but also to all *Brassica* species. The detection of nearly 50 new *P. brassicae*-infested fields during 2012-2015 across several federal states in Germany suggests that clubroot disease maybe more widespread in oilseed rape fields than previously thought. To date, growing resistant cultivars is the most effective and environmentally safe strategy for controlling clubroot (Hirai, 2006; Diederichsen *et al.*, 2009), but sometimes this resistance can be overcome as new pathotypes of the pathogen emerge. In the present study, field trials with natural infection on three different locations in Germany were conducted in 2014 and 2015 to investigate control strategies for improving resistance in susceptible and resistant cultivars by evaluating the effect of different soil amendments at different times during the growing season. Calcium cyanamide (300 kg/ha; 50% calcium oxide) and burnt lime (150 kg/ha) were applied to the soil surface one day prior to the sowing or when the oilseed rape plants had reached the growth stage (BBCH) 11-12. Soil moisture, soil temperature and soil pH at two different depths (15 and 30 cm) were measured at regular intervals over the growing season. Clubroot disease incidence and severity were assessed visually for the development of root galls. Field results in 2014 showed clear differences between the treatments. Changing the time of application had a significant effect ($P \leq 0.05$) on the final severity of the disease. Relative to untreated controls, clubroot incidence and severity were decreased by application of fertilizer at later growth stages. In comparison with calcium cyanamide, burnt lime application has a smaller effect. In 2015, it was decided to increase the amount of burnt lime application to reach the standard field recommended amount. However, no infection or only low infections of *P. brassicae* were observed across field trials and there were no significant effects of any treatment on the incidence of disease.

Key words: *Brassica napus*, clubroot, calcium cyanamide, lime, disease severity

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