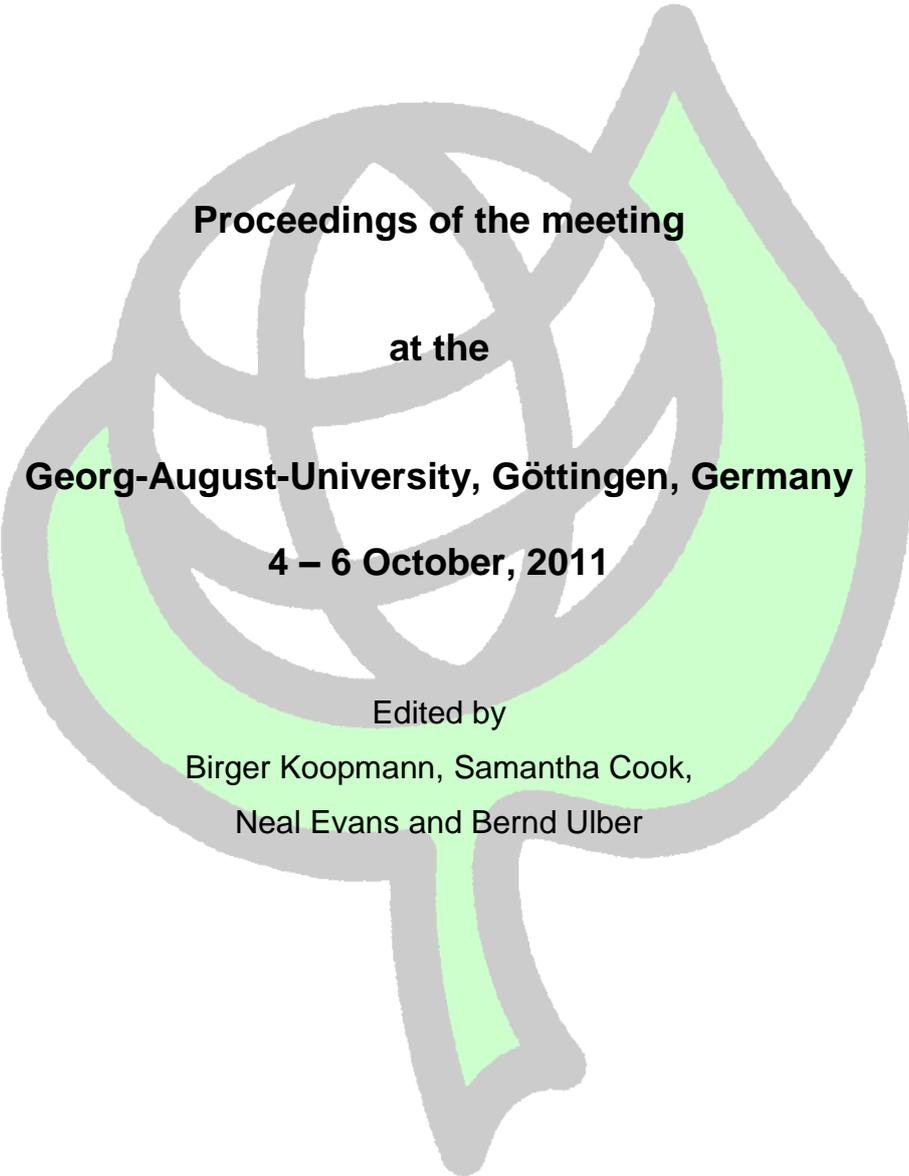


**IOBC-WPRS  
Working Group “Integrated Control in Oilseed Crops”**

**OILB-SROP  
Groupe de Travail “Lutte Intégrée en Culture d’Oléagineux”**



**Proceedings of the meeting  
at the  
Georg-August-University, Göttingen, Germany  
4 – 6 October, 2011**

Edited by  
Birger Koopmann, Samantha Cook,  
Neal Evans and Bernd Ulber

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## Preface

At our last meeting, held in 2008 in Paris, France, we decided to meet next in Göttingen, Germany in 2011. This meeting was hosted by the Georg-August University of Göttingen. Local organisers were Birger Koopmann and Bernd Ulber.

The meeting was well attended by 85 participants from 15 countries including Turkey and Egypt! We returned to our traditional format of having a general session at the start and finish of the meeting, and concurrent Entomology and Pathology subgroup sessions. After the Welcome, Lene Sigsgaard, the IOBC vice-president and the Integrated Control in Oilseed Crops Working Group liaison officer gave an introduction to the IOBC-WPRS highlighting the benefits to membership. She urged individuals to join and drew the groups' attention to a poster presentation about this. Birger Koopmann announced his intention to step down as Convenor of the group at the next meeting in 2013. Dr. Małgorzata Jędrzycka (Institute of Plant Genetics IGR, Poznan, Poland), Dr. Neal Evans (Weather INnovations Incorporated, Stewkley, Buckinghamshire, United Kingdom – formerly Rothamsted Research, Harpenden, UK) and Dr. Xavier Pinochet (CETIOM, Grignon, France) put themselves forward as candidates for this position. Lene Sigsgaard congratulated the Group on announcing this information in plenty of time. Nominations will remain open until October 1<sup>st</sup> 2013. The vote will take place at the next meeting. It was agreed by vote that Sam Cook would remain the Entomology subconvenor to provide some continuation to the WG after the new convenor is elected.

The scientific session was opened with an overview by Manuela Specht (UFOP) on the current situation and future aspects in oilseed rape in Europe. This was followed by an excellent presentation by Prof. Andreas von Tiedemann (Göttingen) on the multidisciplinary research on integrated control in oilseeds done over the past 30 years in Göttingen. This was followed by four general presentations. There were 34 oral presentations in total (17 in each sub group) and 23 poster presentations (11 in the entomology and 12 in the pathology subsections). The meeting of entomology subsection could have been renamed 'Integrated control of pollen beetle in oilseed crops' as all 17 talks and the majority of the poster presentations focussed on pollen beetle related issues. What does this mean? That pollen beetle is *the* key insect pest of oilseed rape? Or that the issues surrounding pyrethroid resistance have wracked-up the political importance of this pest resulting in increased funding opportunities for its study?! For the pathologists, as usual there was a focus on *Sclerotinia* and *Leptosphaeria* with good discussions also on *Verticillium* and clubroot. As usual the focus of the meeting was oilseed rape but there was a welcome change in the form of a presentation on fungal diseases on sunflower presented by Cafer Eken from Turkey.

Drs. Ulber and Koopmann and their team produced an impeccably organized meeting! It was impossible to get lost or to be unsure of what was happening next. Maps of the venue and timetables of busses to the accommodation were prepared and distributed in advance of the meeting and were printed in the abstract booklet for those of us that forgot to bring this information with us! The friendly organizing team were always on hand to help and the technical assistance during the meeting was fantastic. We all enjoyed very much a really interesting guided tour of Göttingen including the medieval Old Town Hall, wine cellars and the impressive Karzer (detention room). This was followed by the Conference dinner, held at La Locanda Italian restaurant to round off a fabulous evening. WELL DONE team Göttingen and VIELEN DANK!

## II

During the meeting we also considered the venue for the next meeting. Dr Michael Eickermann (Centre de Research Public – Gabriel Lippmann, Belvaux, Luxembourg) proposed his Institute as the next venue. He gave a wonderful presentation on Luxembourg and clearly outlined the facilities of the institute – and made the offer too good to refuse! This suggestion was accepted unanimously by the participants. So, we hope to meet you all again in beautiful Belvaux!

*Bis geschwënn zou Lëtzebuerg um Belvaux!*

Sam Cook

Entomology subgroup convenor IOBC Integrated Control in Oilseed Crops

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## **General papers**



## **IOBC-WPRS, Working Group on Integrated Control in Oilseed Crops and membership of IOBC-WPRS**

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The Working Group on Integrated Control in Oilseed Crops (ICOC) is one of 19 Working Groups of the West Palearctic Regional Section (WPRS) of the International Organization for Biological and Integrated Control of Noxious Animals and Plants (IOBC). For more details on other working groups refer to <http://www.iobc-wprs.org/>.

IOBC-WPRS encourages collaboration in promoting feasible and environmentally safe methods of pest and pathogen control. IOBC-WPRS fosters research and practical application, organizes meetings, symposia, offers training and information, especially on biological methods of control, but also on all methods, including chemicals, within an integrated pest management context. Major activities include development and standardization of methods used to test for effects of pesticides on beneficial species, pest and disease damage assessment, modeling in relation to pest and disease management, and the practical implementation of biological and integrated controls for pests and diseases of particular crops.

Working Groups (WG) may publish a proceeding biannually or triennially. Copies of proceedings from WG can be obtained through the publisher of the Bulletin (list and titles of the issues and ordering form can be found on the IOBC-WPRS Website).

**Aims of the WG ICOC:** The working group was established in 1982 by Bent Bromand (Danish Research Centre for Plant Protection) and Christer Nilson (Swedish University of Agriculture) to encourage and coordinate research on integrated disease and pest management systems in oilseed production. Through its regular meetings, it is also intended to serve as a platform to gather and exchange fundamental knowledge and expertise on diseases and pests. Major areas of focus for the group have been:

- Occurrence and distribution of diseases and pests (monitoring work)
- Integrated pest and disease management
- Establishment of damage thresholds
- Development of decision support systems
- Disease resistance
- Use of trap crops for pest control
- Biology of pest and beneficial insects
- Biological control (predators, parasitoids and antagonists)
- Seed pathology in oilseed crops
- Gene technology in oilseed crops – significance, economics and environment

**ICOC convenors / liaison officer:** The Working Group is headed by two convenors: Birger Koopmann is the working group and subgroup convenor Pathology, and Sam Cook is subgroup-convenor Entomology. Sam Cook was elected in 2004 during a working group meeting in Rothamsted following Prof. Ingrid Williams. Birger Koopmann took over from Prof. Volker Paul after election in Copenhagen in 2003. Lene Sigsgaard is the liaison officer of the group and vice president of IOBC-WPRS. She followed Barbara Ekbohm as liaison officer in 2009.

**ICOC Working Group web page:** A web page is available for information on WG-ICOC activities. Meetings are announced, PDFs of abstract booklets are provided and other useful information is available. URL is: <http://www.user.gwdg.de/~iobc/>.

**Membership of IOBC-WPRS:** Members of IOBC-WPRS are individuals, and governmental, scientific or commercial organizations from 24 countries of Europe, the Mediterranean and the Middle East. IOBC-WPRS has four types of memberships: **Individual, Institutional, Supporting** and **Honorary**. Members of IOBC-WPRS are any persons carrying individual or honorary membership, or covered by the membership of their employing institution. Members have access to all working group meetings. Only members can hold any function in the activities of IOBC-WPRS.

Convenors of Working, Study Groups and Commissions are granted free Individual Membership starting with the calendar year immediately after endorsement of their election by the Council, so to honor their contribution.

#### **Membership and benefits:**

- **Individual membership:** 75 €. Includes 10 € for individual membership of IOBC-Global and the possibility to consult and download all recent IOBC-WPRS Bulletins online in the Members' Area of the web site. Also includes a 50-75 € reduction in the registration fee when participating in meetings organized by IOBC-WPRS.
- **Individual membership with subscription to "Biocontrol"** (paper version): 175 €. This includes, in addition to the benefits of an individual membership, a subscription to the international journal "BioControl" for personal use (not for libraries).
- **Supporting membership:** Minimum 350 €. Supporting members are small institutions, libraries, and companies. Supporting membership covers all employees of the unit holding the membership. The supporting membership fees are individually fixed, however the minimum is currently 350 € per annum (including supporting membership to IOBC-Global of 50 € per annum). IOBC-WPRS depends on your contribution to maintain the activities so we ask you to contribute the maximum possible rather than the minimum! Supporting members have the possibility to consult and download all recent IOBC-WPRS Bulletins online in the Members' Area of the web site. Furthermore, they receive one CD with all "IOBC-WPRS Bulletins" published in the year of membership (ca. 10-15 volumes per year).
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- **Institutional Members:** Institutional members are public research / teaching institutions. Their contribution provides a significant part of the funds necessary for IOBC-WPRS to sustain its activities. Please contact any member of the executive committee if your institution wishes to become a member of IOBC-WPRS.

## **Actual situation and future aspects in oilseed rape in Europe**

### **Manuela Specht**

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**Abstract:** The rapeseed crop is of high importance for European agriculture and has recently increased its potential in the EU. In 2011 the EU-27 cultivated nearly 11.2 million ha of oilseeds. Almost 6.8 million ha of this area were cultivated with rapeseed. Rapeseed production is forecast at 19.1 million t. This is in line with the last five years average, but much lower than the production in 2010 (- 6.3%). At EU-27 level the yield forecast is lower than that in 2010 (2.9 t/ha; - 2.4%). Following the good harvest of 2010, German farmers could only achieve very low yields in 2011, which were 23.7% lower than the year before. The most important factor responsible for this failure was caused by very bad weather conditions; heavy rainfall in August and September 2010 resulted in a delay of sowing, followed by a hard and cold winter, then drought during springtime and strong rainfalls during harvest 2011.

The rapeseed oil for food use consists of high-quality lipid acid composition, which is acknowledged by the food and nutrition industry and the consumer in general. Products derived from rapeseed oil are used frequently in a healthy diet and they belong to the group of products with the highest growth rates in food retail sales.

Strong incentives for increased cropping come particularly from the non- food sector; The EU has initiated guidelines for the support of biofuel, in order to establish a proper production for biodiesel. The Renewable Energy Directive (RED) of the European Union set mandatory national targets for renewable energy shares, including 10% renewables in transport (incl. biofuels) by 2020. But RED creates a sustainability regime for biofuels with a strong greenhouse gas saving of at least 35% at present. Germany has been proven to be the leader in the field of biodiesel development.

Forecasts predict a consistent development of the global oilseed sector. Emphasis is on the increasing demand of plant oils in human nutrition, renewable resources for biofuels and bioenergy, as well as their use in animal feed products. As a local oilseed crop with a high yield potential under intensive agronomic cultivation, rapeseed will be an increasing factor of economic importance in European agriculture. The relevance of resulting products of the rapeseed crop in the food, non-food and feed industries will constantly increase.

**Key words:** oilseed rape, rapeseed, European Union Renewable Energy Directive



## Integrated control of pests and diseases in oilseed rape – 30 years of multidisciplinary research in Göttingen

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**Abstract:** Extensive research on oilseed rape (OSR) in Germany began in the early 1980s. Due to new, double low cultivars introduced, the average area of rapeseed cultivation in Germany rapidly grew from 140,000 ha in 1980 to 254,000 ha in 1984 and has reached 1.43 Mio ha at present (2011). As a consequence of increased intensity in production, diseases and pests of OSR became more important. This stimulated research on effective control methods and enhanced especially the demand for cultivars with improved resistance to major diseases. In Göttingen, this challenge was met with several research projects and numerous dissertations. The ultimate aim of these efforts was to deliver a scientific basis for development of various components of integrated control and their integration in suitable IPM systems.

The root collar and stalk disease, also known as ‘black leg’, caused by *Phoma lingam* was among the first diseases of scientific interest. Initial studies were performed in collaboration with Wilhelm Krüger (BBA) on epidemics of the pathogen and resistance screening both in the field (e.g. Wittern *et al.*, 1984) and in greenhouses. This work was followed over years and established close collaborative relationships with breeders through support of their selection efforts in resistance breeding. *Phoma lingam* became a major topic in Göttingen and the aspects addressed were life cycle biology (e.g. Holtschulte, 1992), epidemiology (e.g. Thürwächter *et al.*, 1995) and population structure of the pathogen (e.g. Kuswinanti *et al.*, 1999; Volke, 1999), performed both on the national and European scale (IMASCORE project). In addition, several studies were attributed to effects of reduced tillage on the disease (Voss, 1998; Sievert, 2000).

Based on several years of extensive field experiments to quantify the competition between weeds and OSR, an economic threshold model for weed control was developed in several PhD projects (Gerowitt, 1987; Küst, 1990; Werner, 1996). The state wide applicability was tested and confirmed in cooperative field trials with the German Plant Protection Service.

In the early 1990s a novel disease occurred in practice and gained research interest in Göttingen: *Verticillium*, initially termed *V. dahliae*, nowadays known to be a separate species, *V. longisporum*. Basic knowledge on symptoms, disease development, pathogen biology and detection was gathered in two PhD projects (Holtschulte, 1992; Heppner, 1995). This work was continued and intensified after 2001 with studies on the damage potential of the disease (Dunker, 2006), the potential of biocontrol (Stadler, 2010), potential sources and mechanisms of resistance in the *Brassica* gene pool (Eynck, 2008; Knüfer, 2011) and interactions of *Verticillium* with root-feeding insect pests (Keunecke, 2009). In-depth studies into host-pathogen interactions addressed the signal exchange and the fungal/plant gene expression (Ratzinger, 2008; Riediger, 2008; Weiberg, 2008).

Further interest was in *Sclerotinia* stem rot, particularly in the development of a computer-based forecasting system including economic damage thresholds (SkleroPro), which has been launched to the agricultural practice in 2006 (Dunker, 2006; Koch, 2006).

The OSR Pest Group has been involved in various research projects on the development of *Integrated Pest Management* strategies in Germany since 1988. Basic and applied research on population dynamics of pests, host plant-pest/pathogen-relationships (Dechert, 1999), plant resistance (Eickermann, 2008), economic damage thresholds (Wahnhof, 2000), effects of cultivation techniques (Nuss, 2004) and conservation bio-control of pests (Klingenberg, 1991) has been conducted in order to improve the oilseed rape production systems and reduce the need for insecticide application. Special emphasis was given to the effects of various tillage systems (Voss, 1998; Nitzsche, 1998; Schierbaum-Schickler, 2005) and of pesticide application (Wolf-Schwerin, 1993; Neumann, 2010) on pests and their antagonists. The main focus was on cabbage stem flea beetle, stem weevils, pollen beetle, seed weevil and cabbage root fly as well as on the status and potential of their natural enemies, particularly hymenopterous parasitoids.

**Key words:** tillage systems; pathogens; weeds; interactions; resistance mechanisms; damage potentials; forecasting system; integrated pest management; economic damage thresholds; parasitoids

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## **Winter oilseed rape: a break crop that will ENDURE**

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**Abstract:** The aim of the EC-funded Network of Excellence ENDURE (Project 031499) was to develop a multi-disciplinary and cross-sector approach to the development and implementation of Integrated Pest Management strategies in Europe, creating a coordinated structure that takes advantage of advances in agricultural sciences and technologies. As part of this work we collaborated with colleagues from Denmark and France to analyse current rotational practices and cropping patterns in arable farms. We explored the extent to which different crops and/or crop sequences and emerging IPM technologies could be used to reduce inputs while maintaining profitability and sustainability. Results from the UK highlight the current importance of oilseed rape in the rotation in comparison to other break crops. In addition to being a profitable crop which fits in well with cereals in terms of agronomic practices and on-farm time planning, oilseed rape offers a ‘window of opportunity’ to address UK growers’ most important pest problem, the control of resistant weeds (particularly black grass, *Alopecurus myosuroides*). This paper discusses potential crop rotations for reduced inputs in the UK and the central role of oilseed rape within them.

**Key words:** Integrated Pest Management; cropping practice; sustainable agriculture; crop rotation; oilseed rape; weeds

## **Trait variation in *Brassica napus* – The UK OREGIN diversity demonstration trials**

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**Abstract:** In 2009, the UK OREGIN (Oilseed Rape Genetic Improvement Network) project, funded by Defra, established small-scale Diversity Demonstration Trials over two seasons (2009/10; 2010/11) at Rothamsted Research. The objective of these trials was to observe, sample and collect baseline information describing plant performance and properties of the Diversity Fixed Foundation Set for *B. napus* (BnaDFFS). Within the project, a limited number of component traits (i.e. mineral analyses, architecture and seed composition) affecting yield, harvest index and nitrogen utilisation efficiency (NUE), and their impact on seed composition were assessed. However, a key feature of the OREGIN Diversity Demonstration Trials is that they are available for use by the research community. For the 2009/2010 trial, more than 100 people visited and 78 traits, including pest and disease assessments, were scored collaboratively. Raw data for 2009/10 have been collated and analysed in GenStat<sup>®</sup> providing REML means, scatter plots, distribution of line means, heritability values and outputs for other software. Processing of post-harvest trait data for 2009/10 and further examination of trait variation and patterns of co-variation across all traits is underway. GxE interactions will be investigated with 2010/11 trial data and other relevant datasets. Trait data will be secured in CropStoreDB and made available via the OREGIN website, providing users with the capability to select subsets of BnaDFFS lines for use in more detailed experimental studies of their particular trait(s) of interest.

**Key words:** oilseed rape; diversity; nitrogen use efficacy; pests; diseases; postharvest traits; trait variation, trait covariance

OREGIN Diversity Demonstration Trials: <http://www.oregin.info/resources/trials.php>

BnaDFFS: [www.brassica.info/resource/plants/diversity\\_sets.php](http://www.brassica.info/resource/plants/diversity_sets.php)

CropStoreDB: <http://www.cropstoredb.org/>

## **How to design and assess integrated crop management methods for winter oilseed rape in a network of farmers' field?**

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**Abstract:** Concerns about the adverse impacts of pesticides on the environment and their inevitable negative side-effects on non-target organisms have been growing since the 1960's. For winter oilseed rape (WOSR) (*Brassica napus* L.) in France, the Treatment Frequency Index (TFI) has been increasing since 1994. However, many scientists have been arguing, for more than two decades, that this reliance on chemicals could be considerably reduced by making better use of cultural control. The aim of this paper is to: (1) expose a conceptual scheme illustrating the biotic interaction between pests, diseases and plants and crop management and to describe the possible use of those interactions for WOSR (2) to demonstrate that environmentally friendly crop management for WOSR could be designed and implemented in farmers' fields and assessed with several criteria.

Considering the interaction between disease, weeds or pests with plants, it could be possible: (1) to avoid weeds insect pests or pathogens by shifting the crop and pests cycles, (2) to modify the habitat of pests and thereafter to disturb the behaviour of insects, the contamination of pathogens or the growth of weeds, (3) to reduce the impact of pests. Several examples from the literature were chosen to illustrate these biotic interactions, the impact of crop management and main advantages and limitations of such cultural control. Based on a logical combination of these elementary ways to avoid, disturb or reduce the pests, several environmental friendly crop management tactics (Integrated Crop Management) have been designed following a logical combination of the elementary ways to avoid, disturb or reduce the pests.

We studied 32 plots on 15 fields from six regions with contrasting climatic conditions, distributed all over France from 2005 to 2007. The plots differed in terms of crop management. On each field, at least one integrated crop management and a conventional crop management were tested. Each plot was located on a homogeneous area of the field. Not all the plots were studied every year. Two types of new integrated crop management system (ICM) were tested, according to the results obtained in organic WOSR by Valantin-Morison *et al.* (2007) and Valantin-Morison and Meynard (2004): a strategy based on avoidance of pests and smothering effect on weeds and another based on avoidance of diseases and destruction of weeds before sowing. The choice of the strategy depended on soil depth and soil nitrogen supply. The calculation of TFI (Treatment Frequency Index) was done for herbicides, fungicides, insecticides and molluscicides with the method detailed in Brunet *et al.* (2008). The calculation of energy consumption was split into direct and indirect consumption (Bockstaller *et al.*, 2008).

The mean TFI value obtained for integrated crop management was 3.47, which is 41% less than the mean value for conventional crop management. The reduction of energy use for the integrated crop management system compared to the conventional crop management system was low and achieved 6% with a mean of 10168 MJ/ha (SD = 2303 MJ/ha). Despite a 0.22 t/ha yield reduction (with a max of 0.86 t/ha), the gross margin of ICM was higher than the conventional one, mainly thanks to the reduction of operating costs (for a yield of ICM

ranging from 1.62 to 4.73 t/ha). Agronomic results on disease occurrence and weed competitiveness show that limitation of the number and damage of those pests was possible.

**Key words:** side effects; non-target organisms; treatment frequency index

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# **Entomology papers**



## **Status of insecticide resistance in insect pests of oilseed rape crops in Germany**

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**Abstract:** Pyrethroid resistant pollen beetles (*Meligethes aeneus*) are widely distributed in several European countries. Since 2005 a monitoring programme on oilseed rape pest insects was carried out by the JKI in Germany in order to monitor both pollen beetle resistance and the susceptibility of other oilseed rape pest insects like stem weevils and flea beetles. These latter oilseed rape pest insects are in selected by the use of pyrethroids a similar way to pollen beetles. Laboratory experiments and monitoring activities were conducted using the IRAC Method 11 test design. In all tests,  $\lambda$ -cyhalothrin was used as the active substance representing Type II pyrethroids. In many tests, other active substances were also used. The results of the monitoring showed that the resistance of pollen beetles increased from year to year; in 2010, highly resistant populations dominated in Germany, indeed no susceptible population could be found. For other OSR pest insects (especially *Psylliodes chrysocephala* and *Ceutorhynchus obstrictus*) locations with reduced sensitivity/resistance were detected.

**Key words:** Pyrethroid resistance, *Meligethes aeneus*, *Psylliodes chrysocephala*, *Ceutorhynchus obstrictus*

## **The effects of nitrogen input and flowering on pollen beetle infestation in the OREGIN demonstration trials**

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**Abstract:** The Oilseed Rape Genetic Improvement Network project (OREGIN) has assembled key genetic resources to enable researchers and breeders to explore the relevant gene-pool for enhanced traits to incorporate into breeding programmes. This includes establishing diversity fixed foundation sets for *B. napus* (*BnaDFFS*). The set of founder lines within the *BnaDFFS* was compiled to represent a structured sampling of the genetic diversity across the global *B. napus* genepool, and to encompass winter and spring OSR, swedes, and fodder, forage and salad kales. OREGIN established small-scale demonstration trials to gather baseline information on plant performance and properties of the fixed lines. Assessments were made of component traits affecting yield, harvest index and nitrogen utilisation efficiency (NUE), and their impact on seed composition in two years. In the first year of the project we assessed flowering duration and pollen beetle (*Meligethes aeneus*) infestation on the test lines. There was a wide variation in the start of flowering between the lines. In general, pollen beetles were most abundant on the early-flowering lines. There was little effect of the two nitrogen treatments (low and high) on pollen beetle infestation.

**Key words:** *Brassica napus* gene pool; flowering duration; *Meligethes aeneus*

## Growth rate of pollen beetle populations on different cultivars of oilseed rape

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**Abstract:** The main objective of this project was to determine the effect of oilseed rape cultivar and crop phenology on infestation and population growth of pollen beetle (*Meligethes aeneus* F.) populations. Field experiments were conducted over three years to compare the abundance of adult beetles, their reproduction and population development on four cultivars (cvs) of winter oilseed rape: two cultivars classified as very early flowering and two as late flowering, each represented by open pollinated cvs (NK Passion vs. Favorite) and hybrid cvs (Elektra vs. Titan). Field trials were set up in a complete randomized block design with four replicated plots of each cultivar close to Göttingen.

The number of overwintered pollen beetles was assessed on plants in April at three-day intervals from the early bud stage to full flowering. Samples of buds were collected from all plots and examined for feeding wounds and oviposition holes. The number of eggs and first instar larvae within buds was counted and the number of infested buds was related to the total number of buds available. The abundance and phenology of second instar larvae dropping to the ground for pupation was assessed using water traps. The abundance of adult new-generation beetles emerging from soil was determined by ground-photoelectors.

Depending on the start and duration of the bud and flowering period in relation to the time of beetle immigration, the infestation, reproduction and emergence rate of pollen beetles was affected differently between the tested cultivars. This was particularly obvious in 2008 when the phenology of early and late flowering cultivars clearly differed and the immigration and oviposition of pollen beetles was delayed by cold weather conditions. In the early flowering cultivars the emergence rate of new-generation beetles was lower than those that were late flowering. The population growth rate was significantly higher on cv Favorite than on cv NK Passion. These results were confirmed by semi-field experiments including early and late release of pollen beetles into caged plots.

In 2009 and 2010, when there was only little difference between the phenologies of the four cultivars and colonization of all cultivars by pollen beetles started in the early bud stage, both hybrid cultivars were preferred by overwintered beetles and showed higher larval densities than the open pollinated cultivars. In contrast, a lower percentage of larvae survived to adulthood on hybrid cultivars compared with on open pollinated cultivars, indicating density-dependent effects on larval fitness and on mortality of larvae and pupae in the soil.

Our results suggest that the coincidence between adult immigration and the time of flowering is one of the main factors regulating the annual population growth of pollen beetle in the field. In early and short flowering cultivars, and particularly when beetle immigration is delayed, the time available for oviposition and larval development can be insufficient, resulting in low reproduction rates. Thus, the flowering phenology of oilseed rape cultivars may have a major impact on the growth of pollen beetle populations. Our results confirm earlier observations by Nilsson (1988, 1994).

**Key words:** *Meligethes aeneus*, reproduction, *Brassica napus*, host plant phenology

**Acknowledgement:** This project was funded by the German Federal Ministry of Food, Agriculture and Consumer Protection.

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## The occurrence of different species of pollen beetles in oilseed rape fields

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**Abstract:** Pollen beetles in oilseed rape and their biology were studied from 2008 to 2010. This species is a serious pest of oilseed rape as it causes a significant reduction in yield. It is reported that the species composition of the pollen beetles infesting oilseed rape crops changes during the growing season. *Meligethes aeneus* is resistant to pyrethroids, but it is unknown to what extent, if any, the other species of pollen beetle are resistant. For the effective application of insecticides it is important to know which species are present and when they occur in a crop during a growing season. Besides testing thousands of pollen beetles from different parts of Europe for resistance to different insecticides, the species were identified using relevant keys. These keys are usually produced for academic purposes and include a number of taxa that do not occur on oilseed rape. Therefore, a key was developed for those species that occur only on oilseed rape. This key is based on digital photographs using specimens kindly provided by the Humboldt University (museum of natural sciences, Berlin, Germany), German Entomological Museum (Eberswalde, Germany) and Dr. Jelinek (Prague, Czech Republic).

The analysis revealed that only a single species is dominating on oilseed rape, the true oilseed rape pollen beetle, *M. aeneus*. Reports of seasonal changes in the species composition of pollen beetles on oilseed rape could not be verified. This might be a consequence of the intense and widespread spraying of oil seed rape crops with insecticides, which is likely to kill all the species of pollen beetles that are not resistant to pyrethroids. Nevertheless the identification of the pollen beetles occurring in oilseed rape crops is important as analyses of samples from Switzerland show (Derron *et al.*, 2007). There *M. viridescens*, a species susceptible to pyrethroids, is widely found infesting oilseed rape crops. It is the dominant pollen beetle in this crop in many areas of the country. If such susceptible species are also abundant in other parts of Europe then it may be possible to reduce the application of insecticides in these areas.

**Key words:** pollen beetle, *Meligethes aeneus*, *Meligethes viridescens*, key, identification, insecticide resistance

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## **The hibernation of oil-seed rape pollen beetles (Do beetles resistant to insecticides suffer a higher over-wintering mortality?)**

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**Abstract:** Winter mortality is assumed to be one of the most important factors influencing the population growth of insects. Despite the economic importance and frequent occurrence of mass-flights of the oilseed rape pollen beetle *Meligethes aeneus* (F. 1775), in spring there is little and contradictory information on over-wintering survival for this species. Currently it is not possible to forecast the population density and risk of mass-flights in spring.

Over the last five years, field and laboratory experiments were conducted to broaden the knowledge of the hibernation biology of the pollen beetle in Germany. The questions investigated were:

1. Where do pollen beetles hibernate and in what abundance? Is there a possibility of using such information to predict the risk of mass-flights the following spring?
2. To what extent is population growth influenced by winter mortality?
3. Do beetles resistant to pyrethroids suffer greater over-winter mortality?

In order to characterize pollen beetle hibernation sites, soil samples (0.25 m<sup>2</sup>) were collected monthly from different biotopes from late summer/autumn to the following spring. The beetles in these samples were extracted in the laboratory using a modified MacFadyen apparatus. As it is impossible to determine winter mortality by counting dead beetles, over-wintering survival was assessed based on the number of extractable beetles. Previously reported features characterizing hibernation places were confirmed, i.e. no beetles were found in the litter layer of coniferous forests. Furthermore this study revealed that the distribution of oilseed rape pollen beetles is highly heterogeneous, even at a small scale (i.e. at 1 m<sup>2</sup>) and in suitable biotopes. This heterogeneity and the effort needed to take and extract the samples limits the potential of using this method to forecast the numbers of pollen beetles the following spring. Interestingly the numbers of extractable beetles remained statistically the same throughout hibernation. This suggests that winter mortality is low, at least at the locations and over the periods we studied.

To verify these results, artificially infested samples were dug into the soil at hibernation sites and similarly collected and extracted throughout the winter season. As for the naturally infested soil samples, the winter mortality was low and did not change from autumn to spring. In addition, laboratory experiments were conducted to determine the effect of three different temperature regimes on beetle mortality.

To test the hypothesis that pyrethroid resistant pollen beetles suffer a greater over-wintering mortality than non-resistant beetles, glass vial residue bioassays were conducted using beetles extracted from soil samples at different times during winter. The inner surface of glass vials were coated with different application rates of lambda-cyhalothrin and pollen beetle mortality was assessed after 5 and 24 hours. The mean percentage mortality remained constant throughout winter, suggesting that the pyrethroid resistance in pollen beetles is not linked with reduced winter fitness.

**Key words:** pollen beetle; overwintering, hibernation, forecasting, pyrethroid resistance; fitness trade-offs

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## Ensemble-based analysis of regional climate change effects on the pod midge (*Dasineura brassicae* Winn.) in oilseed rape

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**Abstract:** The impact of regional climate change on the migration of the brassica pod midge (*Dasineura brassicae* Winn.) on crops of winter oilseed rape (*Brassica napus* L.) in the Grand Duchy of Luxembourg was evaluated for the past and projected future time-spans. A threshold-based statistical model for the first and the peak appearance of *D. brassicae* to oilseed rape crops was taken from the literature and combined with selected regional climate change projections of the EU ENSEMBLES project. An ensemble of six regional climate change projections was used to quantify the bandwidths of possible change signals and also to assess the uncertainty associated with these projections. In comparison to the reference period (1961-1990), the onset of the first appearance period was projected to occur between 4.3 (near future, 2021-2050) and 5.3 days (far future, 2069-2098) earlier per decade. Furthermore, the possible time-span of the first migration was prolonged from 20 days (near future) to up to 60 days (far future). The peak appearance period of the pod midge to the field will start significantly earlier (5 days per decade) under future climate conditions. Additionally, the time-span of possible migration will be prolonged from 9 days (near future) to up to 13 days (far future) under projected future climate conditions in comparison to the reference period.

**Key words:** migration period, peak appearance, rapeseed crops, threshold-based model

### Introduction

The brassica pod midge (*Dasineura brassicae* Winn.) (Diptera: Cecidomyiidae) is a severe pest species in winter oilseed rape throughout Europe (Alford *et al.*, 2003). Adults emerge from cocoons in the soil of previous year's fields of oilseed rape and migrate to new oilseed rape crops. Females deposit batches of about 20-30 eggs in young pods, which often have been damaged before by the cabbage seed weevil, *Ceutorhynchus obstrictus* (Ferguson *et al.*, 1995). Due to this infestation, seed weight of damaged pods can be significantly reduced by up to 82% (Williams 2010). In this study, the potential migration period of *D. brassicae* to oilseed rape crops has been investigated under future climatic conditions in Luxembourg based on regional climate change projections from the EU FP6 ENSEMBLES project (van der Linden & Mitchell, 2009). A statistical threshold-based model for migration of *D. brassicae* was taken from the literature (Hansen, 1994) and combined with the regional climate change projections.

### Material and methods

A selection of six regional climate change projections from the EU FP6 ENSEMBLES project (van der Linden & Mitchell, 2009) was used. The selection was based on three criteria: i) only simulation results with a 25 km × 25 km spatial resolution and ii) a temporal coverage up to 2100, iii) the overall ensemble bandwidth in terms of air temperature change signals must be

covered by the selected regional climate models (RCMs) in order to account for many different possible future climate evolutions. The time-span up to 2000 is considered as the ‘control time-span’ (based on observed 20<sup>th</sup> century emission data) followed by the ‘projected time-span’ (based on the Intergovernmental Panel on Climate Change (IPCC), Special Report on Emissions Scenarios (SRES) A1B emission scenario. As the Hadley Centre Coupled Model, version 3 (HadCM3)-driven RCMs temporal coverage ends in November and December 2099, respectively, the far-future time-span is defined from 2069 until 2098 and the near-future time-span is defined from 2021 until 2050. Variables retrieved for this study are daily mean air temperature, daily minimum as well as maximum air temperature. To derive spatial means, all grid cells on the differing model output grids that overlap with the area of Luxembourg were averaged, using a weighting factor per grid element, based on the respective overlap area. Because RCM outputs are systematically biased in comparison to observations, it was necessary to bias-correct them before using them with an impact model (Piani *et al.*, 2010). A linear bias correction approach was used to correct air temperature and is described in detail in Junk *et al.* (2011).

The biological models were chosen according to the availability of the meteorological forcing data (minimum, maximum and mean daily air temperature). We applied threshold-based statistical relationships as defined by Hansen (1994) to describe the first and the peak appearance of the first generation of *D. brassicae* on crops of oilseed rape after emerging from their overwintering sites. This first appearance is based on 80 degree-days (D°) with 8 °C as base temperature, derived from daily mean air temperatures. The peak appearance takes place when 110 D° with 8 °C as base temperature has been reached (Hansen, 1994).

The applied Kolmogorow-Smirnow-Test indicates that all time series show a non-Gaussian distribution. Additionally, the Mann-Whitney U-Test (SigmaStat Ver. 3.0, Erkrath, Germany) was used to test if there are statistically significant differences between the migration periods projected for the difference time-spans.

## Results and discussion

### *Regional climate change*

The spatially averaged annual mean air temperatures for Luxembourg for the selected ENSEMBLES RCMs are given in Figure 1. The multi-model ensemble bandwidth is expressed by the absolute minimum and maximum of the six-member distribution (grey shading). To summarize the projected changes in the near surface air temperature, the multi-model arithmetic mean is shown (thin black line), as well as long term mean values for the three analysed time-spans (thick lines). An increase of the annual air temperature by 1.1 °C (near future) to 3.1 °C (far future) can be expected in comparison to the reference time period.

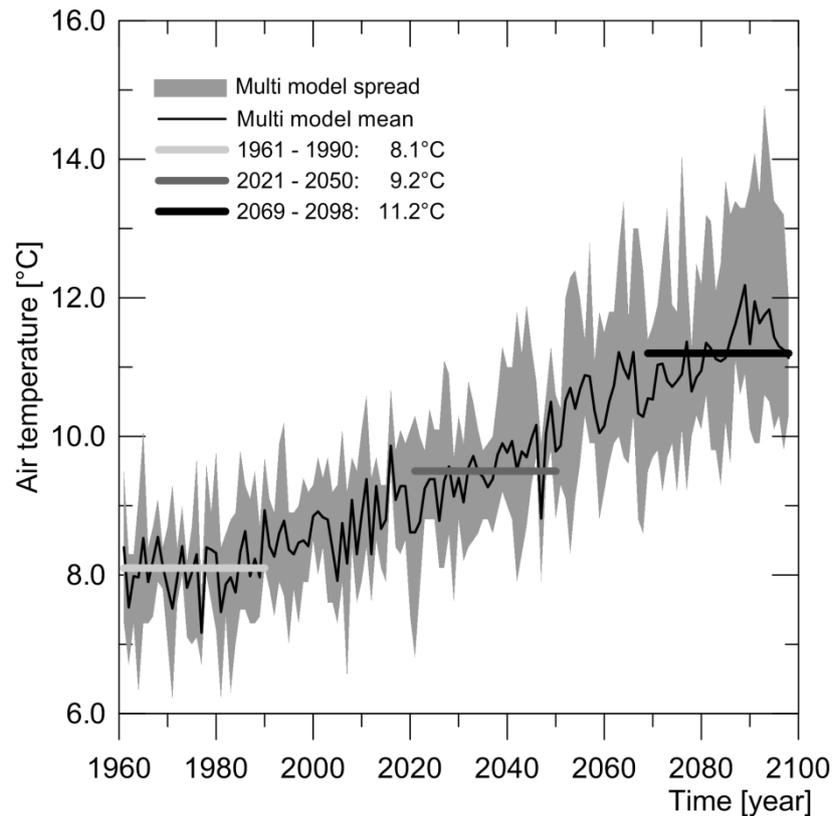


Figure 1. Time-series of annual mean air temperature (spatial mean for Luxembourg): multi model ( $n = 6$ ) spread (grey shading); multi model mean (thin black line) with long-term annual means from 1961 to 1990, 2021 to 2050, and 2069 to 2098 (thick lines).

### ***Migration of brassica pod midge***

The following results are presented as absolute frequency distributions for three different time-spans: the reference period, the near future and far future time-spans. Each frequency distribution consists of the annual event – expressed as day of the year (DOY) – for each year and model, leading to 180 elements per frequency distribution (30 years for each of the six models).

Using the threshold-based model of Hansen (1994), significant differences between the three time-spans for the first appearance of the first generation of *D. brassicae* in crops of oilseed rape can be expected (Figure 2). In the control time-span, first appearance was on average at DOY 116 (26 April). For the two future time-spans, shifts towards earlier dates are expected: DOY 103 (13 April) for the near and DOY 87 (28 March) for the far future (Figure 2). Increasing air temperature can shorten the diapause period (Bale & Hayward, 2010) and may lead to earlier migration and activity (Harrington *et al.*, 2001). A similar effect was detected for the cabbage stem weevil, *Ceutorhynchus obstrictus* (Marsham), with a mean air temperature increase by 3 °C in the future (Olfert & Weiss, 2006). Also, the time-span for the migration of *D. brassicae* was prolonged from 72 days in the reference period to 90 days in the near and 130 days in the far future (Figure 2). A longer flight duration under increasing air temperature was shown also for numerous butterfly species in the analyses of long-term datasets in UK (Roy & Sparks, 2000).

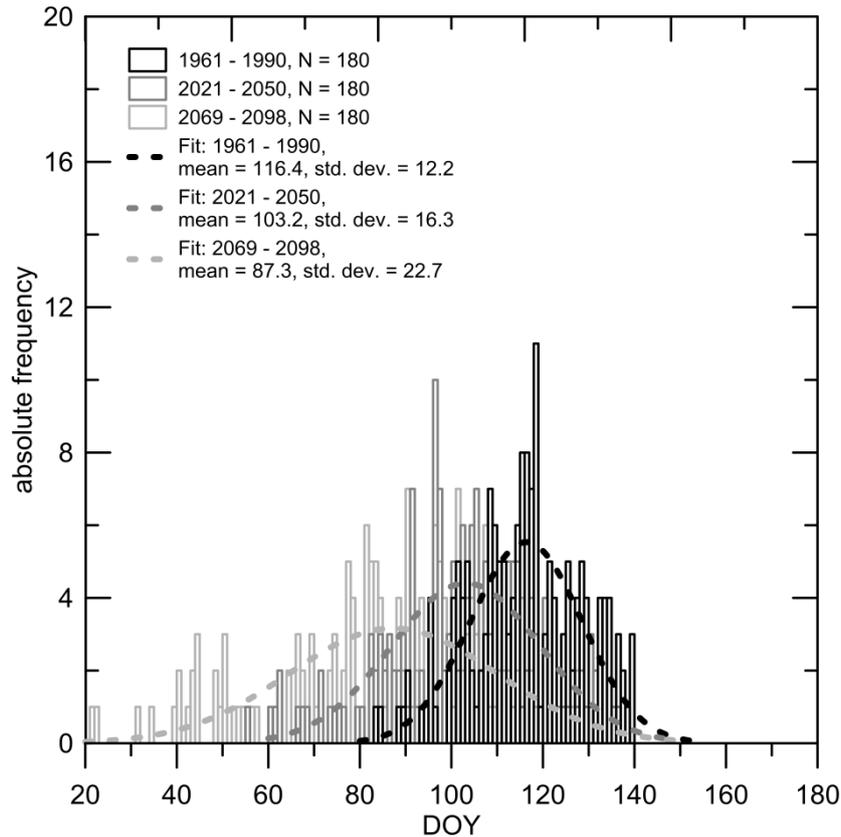


Figure 2. Absolute frequency of the day of the year (DOY) for the first appearance of *Dasineura brassicae* in crops of oilseed rape (according to the model of Hansen, 1994; threshold: 80 D°, with 8 °C base temperature) derived from 30-year time-spans and 6 RCMs (N = 180, all available data combined into a single distribution). Gaussian fits are given for the time-spans from 1961 to 1990, 2021 to 2150, and 2069 to 2098 for an easier interpretation. Arithmetic means and the standard deviations of the distribution functions are also given in the corresponding shades of grey.

Another threshold-based model by Hansen (1994) was used to determine the point in time for the peak appearance of the first generation of *D. brassicae* in the fields (Figure 3). The peak appearance during the reference period was around DOY 143 (23 May). For the two future time-spans, significant shifts towards earlier dates in the near (DOY 133, 13 May) and far future (DOY 118, 28 April) were projected. A similar effect was detected for the cabbage stem weevil, *Ceutorhynchus pallidactylus* (Mrsh.) (Junk *et al.*, 2011) and for several butterfly species on brassicaceous host plants (Roy & Sparks, 2000). The period for the main migration of first *D. brassicae* generation is extended only for the near future projections (9 days). The far future projection only showed a slight shift of 4 days towards an earlier onset (Figure 3), which might be explained by an increasing uncertainty for the far future projection by extreme climatic events (Bale *et al.*, 2002).

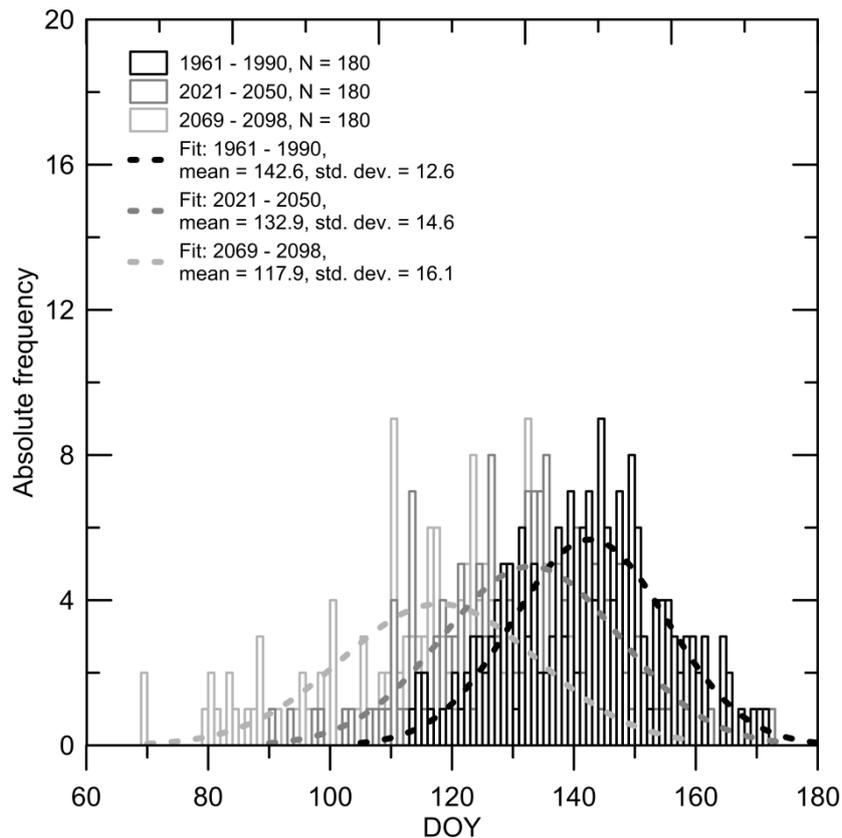


Figure 3. Absolute frequency of the day of the year (DOY) of the peak appearance of *Dasineura brassicae* in crops of oilseed rape (according to the model of Hansen 1994); threshold: 110 D°, with 8 °C base temperature) derived from 30-year time-spans and 6 RCMs (N = 180, all available data combined into a single distribution). Gaussian fits are given for the time-spans from 1961 to 1990, 2021 to 2150, and 2069 to 2098 for an easier interpretation. Arithmetic means and the standard deviations of the distribution functions are also given in the corresponding shades of grey.

Climate change seems to affect the point in time of the first appearance stronger than the peak appearance of the pod midge. The period of first appearance is expected to be considerably prolonged (60 days) in comparison to main flight activity (13 days). In contrast to the peak appearance, the first appearance is triggered by lower temperatures and a lower value of D° that could be reached earlier under climate change conditions. With an extended period of migration, the risk of missing the appropriate time-frame for an insecticide application is therefore higher (Harrington *et al.*, 2001). Further effects of regional climate change can be expected for the future.

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## Population age structure of the cabbage aphid infesting canola plants in Upper Egypt

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**Abstract:** The present studies were carried out throughout the period from 2008-2009 and 2009-2010. The main objectives were to study population age structure of the cabbage aphid infesting canola (*Brassica napus*) in Assiut, Upper Egypt and their natural enemies. Data show that the migration of aphids from their overwintering sites into canola fields occurred after about 23 days (during the third week of December). The population then increased to become 10% of the maximum number after 49 days (during the third week of January). Maximum population density of the cabbage aphid occurred after about 97 days. Therefore, the peak of abundance could be expected around the end of February - beginning of March. After the population reached its highest level it declined and reached 10% of the maximum after 112 days. The population then vanished from the field after 122 days (toward the end of March). The present results indicate that the number of cabbage aphids was significantly higher in the first season 2008-2009 (938.79 aphids/plant), than that of 2009-2010 season (244.77 aphids/plant). The differences in levels of infestation between the seasons might be attributed to the differences in weather factors (temperature, relative humidity) and/or the effect of the common natural enemies in each season.

**Key words:** canola, *Brassica napus* cabbage aphids

### Introduction

Canola (syn. oilseed rape) (*Brassica napus*) is one of the newly introduced oil crops in Egypt planted to contribute to reducing oil shortages. Canola cultivation is recommended for increasing total oil production to bridge the gap between production and consumption of edible oil; the majority of local edible oil production in Egypt comes from cotton seed and does not cover the current needs for oil production. Canola has two types of cropping system, winter and spring (summer) varieties. In Egypt, spring varieties are grown as winter crops (Halaka, 2000).

Canola plantations in Egypt are usually infested with various insect pests, which threaten the yield. The most economically important pests are aphid species including cabbage aphid *Brevicoryne brassicae* L., turnip aphid *Lipaphis erysimi* (Kaltenbach) and green peach aphid *Myzus persicae* Sulzer. They cause direct and indirect problems in the field and these result in economic losses (Ahmed, 1980; El-Kadi, 1980 and Ahmed, 2006).

Cabbage aphid *Brevicoryne brassicae* L. is the most common aphid species infesting canola plantations. (Ryan *et al.*, 1987 and Ahmed, 2006). However, basic information on the population age structure of this aphid species infesting canola plants in Upper Egypt is lacking. Therefore, the present studies were conducted to obtain better knowledge about cabbage aphids infesting canola in the Assiut area.

## Material and methods

The present studies were carried out on the Experimental Farm of Assiut University throughout two successive canola-growing seasons: 2008-2009 and 2009-2010. An area of about half a feddan (2100 m<sup>2</sup>) was cultivated with canola plants (cultivar Pactol). Crops were sown as is usual during the first half of November. Regular conventional agricultural practices were performed as normal but no chemical control was used during the study period. Weeds were removed by hand.

Samples comprising 50 canola plants were randomly collected, placed in transparent polyethylene bags and were returned to the laboratory for counting aphid species and their natural enemies. Samples were taken weekly when the migration of aphids onto the crops from overwintering sites began, and continued until the aphid population and their natural enemies declined to low or undetectable levels. The number of aphids (nymphs and adults) and the associated natural enemies within each species was counted and recorded at each inspection date.

Population age structure of the aphid was described as d1, d2, d3, d4 and d5, where: (d1) = Time (in days) until the first detection of aphid population in the canola field; (d2) = Time (in days) until the detection of 10% of the maximum population density; (d3) = Time (in days) to attain the maximum population density; (d4) = Time (in days) until the disappearance of 90% of the maximum population density and (d5) = Time (in days) until the absolute disappearance of aphids. Duration of each phase (d1-d5) was calculated from December 1st as a starting date when the crop emerged. Coefficients of the daily rate of population increase ( $\alpha_1$ ) and decrease ( $\alpha_2$ ) were calculated according to Freier (1983) where:

$$\begin{aligned}(\alpha_1) &= 0.9 / d_3 - d_2, \\(\alpha_2) &= 0.9 / d_4 - d_3.\end{aligned}$$

Temperature (maximum and minimum) and relative humidity (maximum and minimum) were obtained from a meteorological station located 100 m away from the experimental field site. Data were statistically analyzed using analysis of variance and means were compared according to Duncan's multiple range test.

## Results and discussion

The changes in the population densities of *B. brassicae* on canola plants in the 2009 season are presented in Table 1. Data indicate that the nymphs and adults of the pest were detected on canola plants in relatively low levels (0.93 aphid/plant) during the third week of December when the plants were in the seedling stage. Thereafter, the population tended to increase gradually through January, February and the beginning of March. The maximum level (273.00 aphids per plant) was attained during the first half of March when the plants were in the first stage of ripening. The number of aphids then showed a sharp decrease and nearly vanished from the field during the end of March when the plants were in the middle of the ripening stage.

Data in Table 1 show the seasonal abundance of the cabbage aphid during the 2010 season. The aphid started to appear on canola plants in extremely low numbers (0.20 aphid/plant) at the end of December when the canola plants were in the seedling stage. The population reached a peak of 70.17 aphids per plant during the beginning of March when the plants were in the ripening stage. The population continued with relatively high numbers into

the next week and nearly vanished from the field during the end of March when the plants were in ripening stage.

In both years, the cabbage aphid appeared in the crop towards the end of December and was present to the end of March with a peak numbers occurring during the middle of March when the plants were in the ripening stage (Table 1).

Table 1. Population fluctuation of *Brevicoryne brassicae* infesting canola plants in Assiut, Egypt during the 2008-2009 and 2009-2010 growing seasons.

| Sampling date | Growth stage | Mean no. individuals/plant |        |         |         |
|---------------|--------------|----------------------------|--------|---------|---------|
|               |              | 2009                       | 2010   | Total   | Average |
| Dec. 22       | Seedling     | 0.93                       | 0.20   | 1.13    | 0.56    |
| 28            | Seedling     | 4.17                       | 0.32   | 4.49    | 2.24    |
| Jan. 4        | Seedling     | 2.87                       | 6.47   | 9.34    | 4.67    |
| 12            | Rosette      | 26.23                      | 3.21   | 29.44   | 14.72   |
| 19            | Rosette      | 17.26                      | 10.10  | 27.36   | 13.68   |
| 26            | Bud          | 78.33                      | 36.94  | 115.27  | 57.64   |
| Feb. 5        | Bud          | 137.70                     | 43.64  | 181.34  | 90.67   |
| 12            | Flower       | 61.60                      | 6.94   | 6.54    | 34.27   |
| 23            | Flower       | 166.80                     | 14.92  | 181.72  | 90.86   |
| March 1       | Ripening     | 129.40                     | 21.26  | 150.66  | 75.33   |
| 10            | Ripening     | 273.00                     | 70.17  | 343.17  | 171.58  |
| 19            | Ripening     | 40.50                      | 30.60  | 71.10   | 35.55   |
| Total         | -            | 938.79                     | 244.77 | 1183.56 | 591.78  |
| (%)           | -            | 79.32a                     | 20.68b | 100     | -       |

Table 2. Population age structure of *B. brassicae* (in days)\* and maximum number of individuals per plant on canola during the 2008-2010 growing seasons in Assiut, Egypt.

| Season      | (d1)         | (d2)      | (d3)         | Max. No.      | (d4)          | (d5)          |
|-------------|--------------|-----------|--------------|---------------|---------------|---------------|
| 2009        | 22           | 52        | 100          | 273.00        | 110           | 122           |
| 2010        | 23           | 46        | 93           | 70.17         | 115           | 121           |
| <b>Mean</b> | <b>22.50</b> | <b>49</b> | <b>96.50</b> | <b>171.58</b> | <b>112.50</b> | <b>121.90</b> |

\* (d1) = time (in days) from crop emergence until the first detection of aphids; (d2) = time until the detection of 10% of the maximum population density; (d3) = time to attain the maximum population density; (d4) = time until the disappearance of 90% of the maximum population density and (d5) = time until the absolute disappearance of aphids.

The population growth of this aphid species could be described by dividing this population into five categories as shown in Table 2. The migration of aphids from the overwintering site into the canola field (d1) occurred after a mean of 23 days (during the third week of December). The population then increased to become 10% (d2) of the maximum number after a mean of 49 days (during the third week of January). Maximum population

density of the cabbage aphid occurred after about 97 days. Therefore, the peak abundance could be expected around the end of February - beginning of March. After the population reached its highest level it generally declined and reached 10% of the maximum (d4) after 112 days. The population then vanished from the canola field (d5) after 122 days (toward the end of March). Data in Table 2 also shows that the duration from the first appearance of the cabbage aphid population (d1) up to disappearance (d5) was about 17 weeks. The most active period of population growth (from d2 to d4) was about 9 weeks. Rate of population increase ( $\alpha_1$ ) was about 0.01/aphid/day and the rate of decrease ( $\alpha_2$ ) was 0.03/aphid/day (Table 3).

Table 3. Coefficient of daily rate of population increase ( $\alpha_1$ )\* and decrease ( $\alpha_2$ )\* of *B. brassicae* and duration of each population age (in days).

| Seasons     | (d3-d1)   | ( $\alpha_1$ ) | (d3-d2)      | (d4-d3)   | ( $\alpha_2$ ) | (d5-d3)   | (d5-d1)   | (d4-d2)      |
|-------------|-----------|----------------|--------------|-----------|----------------|-----------|-----------|--------------|
| 2009        | 78        | 0.019          | 48           | 10        | 0.09           | 22        | 100       | 58           |
| 2010        | 70        | 0.019          | 47           | 22        | 0.04           | 28        | 98        | 69           |
| <b>Mean</b> | <b>74</b> | <b>0.01</b>    | <b>47.50</b> | <b>16</b> | <b>0.03</b>    | <b>25</b> | <b>99</b> | <b>36.50</b> |

\* Coefficients of the daily rate of population increase ( $\alpha_1$ ) and decrease ( $\alpha_2$ ) were calculated according to Freier (1983) where: ( $\alpha_1$ ) =  $0.9 / d_3 - d_2$  and ( $\alpha_2$ ) =  $0.9 / d_4 - d_3$ . [(d1) = time (in days) from crop emergence until the first detection of aphids; (d2) = time until the detection of 10% of the maximum population density; (d3) = time to attain the maximum population density; (d4) = time until the disappearance of 90% of the maximum population density and (d5) = time until the absolute disappearance of aphids].

Table 1 shows that the numbers of aphids were significantly higher in the first season 2008-2009 (mean total of 938.79 aphids/plant), than that of the 2009-2010 season (244.77 aphids/plant) (see also Tables 4 & 5). The differences in levels of infestation between the seasons might be attributed to the differences in weather factors (e.g. temperature, relative humidity) and/or the effects of natural enemies in each season. The general means of temperature ranged from 16.51 - 22.70 °C in the first season (Table 4) and from 13.00 - 20.19 °C for the second season (Table 5). Relative humidity ranged from 48.43 - 56.43% in 2009 (Table 4) and 41.29 - 48.14% in 2010 (Table 5).

This investigation focuses on the seasonal abundance of the cabbage aphid during two growing seasons in relation to plant growth stage, temperature, relative humidity, effective temperature and natural enemies. It could be generally concluded that the population of the pest appeared in low numbers during the third week of December. At this time, the plants were in the seedling stage (plant age of 45 days) and temperature ranged from 7.57 - 28.43 °C, relative humidity ranged from 15 - 84% and day-degrees ranged from 59.29 to 95.34 DD. Predators were not recorded during this phase. Few parasitized aphids (aphid mummies) were observed during the initial infestation of the canola crop. The population of cabbage aphid increased markedly as plant growth stage progressed toward maturity and the maximum population densities of aphids occurred when the plants were in the ripening stage. At this point plant age was on an average 115 days. This period (mid-March) coincided with the maximum temperatures which ranged from 22.14 - 25.57 °C; maximum RH ranged from 66.86 - 80.71% and an average effective temperature of 764.39 day-degrees. These conditions seem to be the favorable range for the reproduction and multiplication of the cabbage aphid. This period coincided with the end of the ripening stage of the canola plants. The number of predators and mummified aphids progressively increased to exhibit a peak as the aphid

populations declined. However, the eventual decline of aphid populations later in the growing season results probably from a combination of rapid drop in the suitability of the crop at this time, accompanied by much alate emigration as well as due to the action of the aphids' natural enemies.

Table 4. Population densities of *B. brassicae* infesting canola in relation to selected abiotic and biotic factors in Assiut, Egypt 2009.

| Sampling date | No aphids / plant | Temp. (°C) |       |       | R.H. (%) |       |       | Natural enemies |       |
|---------------|-------------------|------------|-------|-------|----------|-------|-------|-----------------|-------|
|               |                   | Max.       | Min.  | Avg.  | Max.     | Min.  | Avg.  | Paras.          | Pred. |
| Dec. 22       | 0.93              | 28.43      | 13.14 | 20.79 | 84.00    | 19.57 | 52.00 | 0.10            | 0     |
| 28            | 4.17              | 25.21      | 11.79 | 18.37 | 80.29    | 31.00 | 56.43 | 0.23            | 0     |
| Jan. 4        | 2.87              | 22.57      | 10.43 | 16.51 | 86.29    | 22.29 | 54.43 | 0.55            | 0     |
| 12            | 26.23             | 26.14      | 8.93  | 17.54 | 87.29    | 19.57 | 53.57 | 2.58            | 0     |
| 19            | 17.26             | 27.93      | 11.79 | 19.87 | 83.43    | 24.71 | 55.00 | 1.26            | 0     |
| 26            | 78.33             | 26.71      | 11.86 | 19.29 | 83.14    | 25.43 | 54.57 | 16.04           | 0     |
| Feb. 5        | 137.70            | 29.00      | 11.50 | 20.26 | 79.86    | 21.43 | 51.00 | 13.90           | 0.5   |
| 12            | 61.60             | 30.29      | 10.79 | 20.50 | 86.86    | 19.29 | 53.43 | 3.90            | 0.2   |
| 23            | 166.80            | 29.14      | 11.21 | 19.77 | 73.71    | 17.57 | 45.86 | 5.20            | 0.5   |
| March 1       | 129.40            | 27.00      | 11.00 | 19.01 | 76.43    | 20.14 | 48.71 | 4.40            | 0.3   |
| 10            | 273.00            | 25.57      | 10.50 | 18.01 | 80.71    | 22.57 | 51.14 | 9.80            | 0     |
| 19            | 40.50             | 31.79      | 13.57 | 22.70 | 78.29    | 19.57 | 48.43 | 0.00            | 0     |

Table 5. Population densities of *B. brassicae* infesting canola in relation to selected abiotic and biotic factors in Assiut, Egypt 2010.

| Sampling date | No aphids / plant | Temp. (°C) |       |       | R.H. (%) |       |       | Natural enemies |       |
|---------------|-------------------|------------|-------|-------|----------|-------|-------|-----------------|-------|
|               |                   | Max.       | Min.  | Avg.  | Max.     | Min.  | Avg.  | Paras.          | Pred. |
| Dec. 22       | 0.20              | 23.71      | 7.57  | 15.64 | 71.86    | 15.00 | 43.57 | 0.10            | 0.00  |
| 28            | 0.32              | 23.14      | 9.57  | 16.36 | 76.86    | 19.29 | 48.14 | 0.23            | 0.00  |
| Jan. 4        | 6.47              | 21.86      | 5.57  | 13.71 | 74.57    | 17.57 | 46.29 | 0.55            | 0.00  |
| 12            | 3.21              | 25.00      | 5.71  | 15.36 | 76.43    | 17.29 | 47.71 | 2.58            | 0.00  |
| 19            | 10.10             | 22.86      | 7.57  | 15.21 | 75.71    | 14.86 | 45.00 | 1.26            | 0.00  |
| 26            | 36.94             | 19.71      | 6.27  | 13.00 | 75.00    | 26.00 | 51.00 | 16.04           | 0.00  |
| Feb. 5        | 43.64             | 21.86      | 4.43  | 13.86 | 74.14    | 22.29 | 45.86 | 13.90           | 0.50  |
| 12            | 6.94              | 17.50      | 4.21  | 10.87 | 70.57    | 13.00 | 41.86 | 3.90            | 0.25  |
| 23            | 14.92             | 25.50      | 8.14  | 16.83 | 70.57    | 11.71 | 41.29 | 5.20            | 0.50  |
| March 1       | 21.26             | 28.57      | 11.71 | 20.19 | 75.57    | 13.57 | 44.71 | 4.40            | 0.25  |
| 10            | 70.17             | 22.14      | 8.29  | 15.21 | 66.86    | 19.57 | 43.29 | 9.80            | 0.00  |
| 19            | 30.60             | 28.29      | 8.86  | 18.57 | 73.57    | 13.14 | 43.43 | 0.00            | 0.00  |

Three species of aphids were detected on canola plants in the duration of our study: Cabbage aphid, *Brevicoryne brassicae* L., Green peach aphid, *Myzus persicae* (Sulz.) and Turnip aphid, *Lypaphis erysimi* (Kaltenbach). Previous studies in Egypt and abroad report that canola plants are subjected to attack by these aphid species (Sadivay and Vasak, 2002; Kakmakar, 2003 and Ahmed, 2006).

The cabbage aphid has become one of the three primary pests of winter-seeded canola in Egypt. Cabbage aphid pressure just prior to and during bloom aborts flower buds, deforms developing pods, and generally saps vigor from plants resulting in yield losses of up to 40% in untreated fields. Colonies of more than 300 aphids per raceme are common each season.

The cabbage aphid is a pest of many cruciferous crops and is distributed throughout all the temperate and warm temperate regions of the world. This aphid is considered one of the most damaging and consistently present pests on cabbage crops (Theunissen, 1989). It causes direct damage which may induce plant deformation (Ibbotson, 1953; Oatman and Platner, 1969), and indirect damage caused either by honeydew or by transmission of viruses. The cabbage aphid is a vector of 20 virus diseases in a large range of plants (Chan *et al.*, 1991).

In general, the cabbage aphid appeared in the period from the end of December up to the end of March with a peak number during the middle of March when the plants were in the ripening stage. Our results provide information about aphid species infesting canola in Egypt which may help to achieve a successful control programme for this damaging pest.

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# **Integrated Pest Management**



## Effects of different management systems on pest infestation of oilseed rape in Croatia, Germany and Serbia

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**Abstract:** While Germany is one of the largest producers of oilseed rape (OSR) in Europe (1.6 million ha), OSR production is of increasing importance in Western Balkan Countries (WBC's) where 20% of the area cultivated with arable crops is suitable for OSR production. Problems of OSR cropping are overfertilization with nitrogen, intensive tillage and the over-use of pyrethroids and phosphoric acids which have led to pest resistance. Environmentally friendly production techniques are demanded. This EU-project (SEE-ERA-NET.PLUS) focusses on the within-field biodiversity. Besides classical aspects (species richness/composition) this project comprises functional aspects like biocontrol of pests by predators (Carabidae, Staphylinidae, Araneae), and turnover of plant residues by decomposers (earthworms). In Croatia and Serbia research on OSR is recently focused on general crop production and pest control. Nothing is known about within-field biodiversity of functional groups like epigeic predators and decomposers. Organic OSR production has been introduced for the first time to Croatia and Serbia through this project.

In a 2-year field experiment in each country, the impact of three differently managed OSR fields (conventional, advanced integrated, organic) on pests and on the biodiversity and fitness of predators and decomposers are investigated. The systems differ in tillage, fertilizer and pesticide input, weed control, row space and application of *Brassica campestris* trap-crop-strips and are integrated in a crop rotation with winter wheat. The fields are covered by a grid of sampling points using different methods to assess predators (pitfall traps, endogaic pitfall traps, emergence traps), decomposers (soil samples) and pests (bud/pod/stem samples). The experimental work will constitute a demonstration of possible ecological approaches in OSR production and thus, will give impulses towards research activities which focus more on aspects of functional biodiversity. The project started in October 2010. The first results on pest infestation are will be presented here.

In autumn, serious pest attacks were only recorded by *Athalia rosae* in Serbi, October 2010. The organic OSR recovered after severe *A. rosae*-infestation. *Ceutorhynchus napi* and *C. pallidactylus*, which usually immigrate into OSR-fields in Germany in spring, occurred in Croatian and Serbian OSR-fields as early as November. In all countries, trap crop strips (in integrated and organic OSR) with Perko (*Brassica campestris* x *B. pekinensis*) contributed to pull pests from the oilseed rape field stand, but its effect is limited if the pressure of pest insects exceeds a certain level and if there is no option to conduct an insecticide application. Thus, its function is optimal in combination with integrated crop management. In Serbia organic OSR showed a great potential to recover regardless of an extreme level of pest attack, especially of *Ceutorhynchus* stem weevils.

In summary, the first results indicate that despite climatic differences in the three countries, the soil quality and conditions as well as the surroundings of OSR fields are key factors to determine the level of pest attack.

**Key words:** oilseed rape; insect pests, Croatia, Serbia, conventional, integrated, organic cropping

## **Suitability of different cultivars of turnip rape as trap crops for integrated control of major pests on winter oilseed rape**

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**Abstract:** Turnip rape (*Brassica rapa*) has been reported to be a suitable trap crop for the management of insect pests in oilseed rape (*Brassica napus*). Due to its high attractiveness to major pests it has potential to divert pest insects from the main crop, thereby preventing damage to oilseed rape.

In the years 2008-2010, two types of field experiments were conducted in order to identify the most suitable turnip rape cultivar for trap cropping and to assess the effect of trap crop perimeter strips on the spatial within-field distribution of pests. In randomised field plot experiments, one oilseed rape cultivar ('Robust'), one oilseed turnip rape cultivar ('Largo') and two cover crop turnip rape cultivars ('Perko', 'Malwira') were screened for their attractiveness to the most important insect pests. In the other field experiments, perimeter strips with alternating plots of turnip rape ('Largo') and oilseed rape ('Robust') (6m width x 60m length) were sown along two opposite edges of an oilseed rape field. The effect of turnip rape and oilseed rape perimeter plots on the level of pest infestation within the adjacent oilseed rape main crop (10, 20 and 30 m from perimeter plots) was investigated in spring and summer.

Cabbage stem flea beetle (*Psylliodes chrysocephala*) and pollen beetle (*Meligethes aeneus*) significantly preferred all tested turnip rape cultivars to oilseed rape, measured as number of flea beetle larvae per plant, number of pollen beetle adults in flowers and damaged buds, respectively. Only the pollen beetle discriminated between different cultivars of turnip rape and significantly preferred 'Malwira' to 'Largo' and 'Perko'. In contrast, the cabbage seed weevil (*Ceutorhynchus assimilis*) preferred oilseed rape to turnip rape: pods of oilseed rape showed a significantly higher level of infestation by weevil larvae than turnip rape pods.

Similar to the results of the cultivar screening, perimeter plots of turnip rape were preferred by most insect pests compared to oilseed rape. However, the higher aggregation of cabbage stem flea beetle and pollen beetle in the turnip rape border plots did not result in lower pest infestation in the adjacent oilseed rape main crop. Further, grain yield of the main crop did not differ significantly between plots bordered by turnip rape and oilseed rape.

The use of the turnip rape cultivar 'Malwira' may improve the efficacy of the perimeter strips against pollen beetle.

**Key words:** Cabbage stem flea beetle (*Psylliodes chrysocephala*), pollen beetle (*Meligethes aeneus*), seed weevil (*Ceutorhynchus assimilis*), *Brassica rapa*, trap crop

## Use of plant defence-inducing chemicals in ‘push-pull’ pest control strategies in oilseed rape

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**Abstract:** The push-pull strategy deploys stimuli from plant compounds or semiochemicals to deter or ‘push’ colonising insects away from a harvestable crop and simultaneously attracts them (‘pull’) to a sacrificial or trap crop. We investigated the use of semiochemicals, namely plant inducers, and trap cropping in a push-pull pest control strategy for pollen beetles in oilseed rape. Previous work has shown that application of methyl jasmonate (MeJa) to Brassicas induces the production of indolyl glucosinolates (defence compounds which help protect Brassicas from generalist herbivores) and that application of salicylic acid (SA) induces production of alkyenyl glucosinolates (these defence compounds break down upon plant damage to produce volatile isothiocyanates which are highly attractive to Brassica specialist pests). The hypothesis that application of MeJa to an oilseed rape crop would make it less attractive to pests, in particular generalist pests (e.g. the aphid *Myzus persicae*), and that application of SA to turnip rape trap crops would make them more attractive, particularly to the specialist pests (e.g. pollen beetles and *Brevicoryne brassicae* and *Lipaphis erysimi* aphids), was tested in laboratory and field experiments. No significant effect of the plant-inducing chemicals was found on the host-selection behaviour of the test insects in laboratory experiments. However, oilseed rape treated with a range of concentrations of MeJa was generally less attractive, and turnip rape treated with SA was generally more attractive, than control plants as expected. In field trials, plots of oilseed rape treated with MeJa and bordered with a turnip rape trap crop treated with SA was infested with fewer beetles than control plots, but due to large variation, differences were not significant. Experiments to further explore these trends are necessary.

**Key words:** push-pull, methyl jasmonate, salicylic acid, glucosinolates, plant defence inducing chemicals, pollen beetles, aphids, *Brassica napus*

## **Effect of turnip rape trap crops on the infestation of winter oilseed rape by pollen beetle**

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**Abstract:** Pollen beetle, *Meligethes aeneus* F. has developed widespread resistance to pyrethroid insecticides in recent years. To reduce the current extensive use of insecticides, alternative control strategies are needed which have potential to keep pollen beetle infestation on crops of oilseed rape below threshold levels. We investigated the potential of winter turnip rape, *Brassica rapa* (cv. 'Perko') for protection of the main crop of winter oilseed rape, *Brassica napus*, from infestation by pollen beetle. The effect of the turnip rape trap crop on the abundance, spatio-temporal distribution and damage of pollen beetles on the oilseed rape main crop was examined over three years in multiple field experiments at two locations close to Rostock and Göttingen in Northern Germany. In each field, replicated plots of oilseed rape (50 m wide x 60-100 m long) were bordered at one edge either by a turnip rape strip (6 m wide x 60-100 m long) or by oilseed rape; trap crop plots alternated with control plots. In addition, the effect of targeted insecticide applications on these border strips on pollen beetle abundance and distribution on the main crop was investigated.

The turnip rape trap crop borders attracted significantly higher numbers of pollen beetles than the oilseed rape borders, as long as the main crop of winter oilseed rape was in the bud stage. However, in all experiments the preference of adult beetles for turnip rape did not result in lower numbers of pollen beetles in the adjacent main crop. This might be due to the phenology of the turnip rape inflorescences which developed only 3-4 days earlier than the inflorescences of oilseed rape. Numbers of pollen beetles were reduced in border plots that were directly sprayed with insecticides. However, insecticide treatments targeted to the border strips had no significant effect on the abundance and spatial distribution of pollen beetles in the adjacent oilseed rape main crop, as compared to oilseed rape plots bordered by untreated strips. Feeding damage of pollen beetles on oilseed rape was not significantly different between all treatments.

**Key words:** Pollen beetle *Meligethes aeneus*, turnip rape *Brassica rapa*, trap crop

**Acknowledgement:** This project was financially supported by the German Federal Ministry of Food, Agriculture and Consumer Protection.

## Mixed cropping with turnip rape and natural insecticides: Results of field and laboratory trials on pest control in organic winter oilseed rape

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**Abstract:** The demand for organic winter oilseed rape is steadily increasing. Yet in Germany, for example oilseed rape cultivation is negligible with a maximum cropping area of 4,000 ha. One important reason for this is the occurrence of insect pests, including the cabbage stem flea beetle (*Psylliodes chrysocephala* L.), the rape stem weevil (*Ceutorhynchus napi* Gyll.), the cabbage stem weevil [*Ceutorhynchus pallidactylus* (Marsh.)], the pollen beetle (*Meligethes aeneus* F.), the cabbage seedpod weevil (*Ceutorhynchus assimilis* Payk.) and the brassica pod midge (*Dasineura brassicae* Winn.). Pest-related yield losses – up to total loss of the crop – make the cultivation of organic winter oilseed rape an incalculable risk. Currently, the regulatory options for organic oilseed rape are totally inadequate to address this threat. Against this background, a three-year research project on pest control in organic winter oilseed rape was established. It was coordinated by the Julius Kühn-Institut and sponsored by the Federal Organic Farming Scheme and other forms of sustainable agriculture (BÖLN). The focus of this study was to assess the pest-regulating effect of an oilseed rape-turnip rape mixed cropping system (ratio 9:1) in comparison to oilseed rape in pure stand on three test sites. Further, natural insecticides and self-produced natural insecticide solutions – in accordance with § 6a of the Plant Protection Act – to regulate the stem weevils (*Ceutorhynchus* spp.) and the pollen beetles (*Meligethes aeneus* F.) were tested in laboratory and field experiments. Insecticides tested against the stem weevils were Spruzit<sup>®</sup> Neu (a. i. 18.36 g<sup>-1</sup> natural pyrethrum) and SpinTor (a. i. 480 g l<sup>-1</sup> Spinosad). Against the pollen beetle, SpinTor, Agrinova-Milbenfrei [a. i. Kieselguhr (SiO<sub>2</sub>)] with sunflower oil, Surround<sup>®</sup> (a. i. Kaolin) with canola oil or pine oil, Edasil<sup>®</sup> (a. i. calcium bentonite) and sunflower oil were used. In addition Quassia (a. i. Quassin) and Biscaya<sup>®</sup> (a. i. 240 g l<sup>-1</sup> Thiacloprid) were applied in laboratory experiments. As a mechanical plant protection measure, a beetle collecting machine was tested. For optimum wetting of the plants with a mineral-powder film various quantities of a wetting agent were tested under laboratory conditions.

The increased attractiveness of turnip rape as compared to the oilseed rape crop was confirmed for all pest insects with the exception of the pod pests. Compared to the fields with oilseed rape in pure stands, the mixed-cropping system showed a significantly greater abundance of pests. This resulted in a sometimes significantly greater pest infestation of the oilseed rape plants in the mixed-cropping system. Furthermore, the plots with the mixed-cropping system often had a significantly lower grain yield. Therefore, the cultivation of a rape-turnip rape mixed cropping system, cannot be recommended.

The use of natural pyrethrum and Spinosad resulted in significantly higher mortality rates of the stem weevils in laboratory tests. By contrast neither an effect on the infestation level nor on the amount of plant damage could be detected under field conditions when the agents were used. In the regulation of the pollen beetles, Spinosad was the sole agent under field conditions to demonstrate constant and high efficiencies of up to 100%. This was confirmed

in laboratory experiments. However, it should be noted that this agent is not approved for oilseed rape and is highly toxic to bees, so would be contrary to the principles of organic farming.

Sprayed mineral powder or SiO<sub>2</sub> had only a slight effect on the regulation of the pollen beetles. Generally the formulation of the hand-made phytosanitary broth and its multiple and repeated application are crucial factors to achieve a uniform wetting of the plants. Only by multiple applications was it possible to achieve decreased feeding activity of the pollen beetles under laboratory conditions. From an economic perspective, however, multiple applications are inefficient.

For reasons of practicality, rock-dusted flour and the beetle-collecting machine are not suitable for pollen beetle regulation. As an alternative agent for the regulation of the pollen beetle Quassin is just as inappropriate as the active ingredient Azadirachtin, or *Bacillus thuringiensis* (*B.t.t.*). With the exception of one Spinosad application, no protection measure provided a yield benefit. Factors such as nitrogen deficiency or weeds seem therefore to more frequently limit yield than the infestation of pests under slight to moderate pest infestation levels. The probability to realize an economic surplus through crop protection measures increases with growing nutrient supply of the oilseed rape crop and decreasing weed competition. Adequate crop protection strategies, however, have not yet been developed.

**Key words:** organic production, oilseed rape-turnip rape mixed cropping, natural pyrethrum, Spinosad, SiO<sub>2</sub>, Kaolin, calcium bentonite, Quassin, wetting agents

## Developing an integrated pest management strategy for pollen beetles in winter oilseed rape – a UK Defra SA LINK project (LK09108) (HGCA RD-2007-3394)

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**Abstract:** Pollen beetles (*Meligethes aeneus*) are a serious pest of oilseed rape crops Europe. Resistance to the major group of insecticides used to control them – the pyrethroids – is now widespread. Integrated pest management (IPM) strategies are urgently needed to reduce the number of insecticide treatments to lessen selection pressure for resistance and to protect the environment. The UK Sustainable-Arable LINK project LK09108 ‘Developing an integrated pest management strategy for pollen beetles in winter oilseed rape’ has been funded by Defra and AHDB-Home Grown Cereals Authority (RD-2007-3394) to address this need. The project consortium has 2 academic partners (Rothamsted Research and Imperial College London) and 13 industrial partners comprising representatives of the agrochemical industry (Bayer CropScience Ltd. and Syngenta Crop protection UK Ltd.), Crop Advisers (Association of Independent Crop Consultants), oilseed rape plant breeding companies (Syngenta Seeds Ltd., Elsoms Seeds Ltd., KWS UK Ltd., Limagrain UK Ltd., Monsanto UK Ltd., Saaten-Union UK Ltd.), the oilseeds levy board (AHDB-HGCA), and specialists in trap manufacture (Oecos Ltd), decision support (proPlant GmbH) and software for bioscience (VSN International Ltd. – the makers of GenStat). Together we are developing an IPM strategy based on three tactics: (i) improved monitoring, (ii) risk assessment and (iii) novel crop management i.e., trap cropping.

**Key words:** *Meligethes aeneus*; monitoring; risk assessment; trap cropping



## **The potential of silicate rock dust to control pollen beetles (*Meligethes* spp.)**

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**Abstract:** In organic agriculture, treatments with rock dusts for soil amendment, disease prevention, or insect control have a long tradition. Clinoptilolite (a naturally occurring zeolite) was tested against pollen beetle *Meligethes* spp. in organic and IPM oilseed rape fields in order to find a control strategy compatible with the guidelines for organic and IPM agriculture in Switzerland. Dust and spray applications were evaluated in several large-scale field trials from 2008 to 2011. Dust applications using 300-750 kg/ha pulverized Clinoptilolite (particle size < 0.1 mm; product Klinofeed, available from Unipoint, Switzerland) were applied using a drop-box fertilizer spreader or a Vicon pendulum spreader. For spray applications the same Clinoptilolite (product Klinospray) with a particle size of 0.017 mm was applied at rates of 30-50 kg/ha with a wetting agent (Heliosol, 2 l/ha; 600 l water/ha) using a standard field crop sprayer. Two or three applications were conducted during the inflorescence stage (BBCH 51-57) depending on the rate of development of the oilseed rape plants.

Under the dry and sunny weather conditions in 2009 and 2010, the treatments significantly reduced the number of pollen beetles by 50 to 80% until seven days after treatment (BBCH 51-54). Under the rainy weather conditions in 2008, no reduction of pollen beetles was observed. However, pollen beetles in treated plots showed a lower activity compared to beetles from the untreated control plots. In all experimental years flowering was visibly more intense in the treated than control plots. Pod setting on the main raceme was significantly increased in the treated plots. The yield was significantly increased by 23% in the experiments conducted under IPM conditions in 2010. However, no yield increase was observed in 2008 and 2009 under organic agricultural conditions.

**Key words:** *Meligethes* spp., organic agriculture; stone meal; rock dust; clinoptilolite; zeolite

### **Introduction**

In organic agriculture, methods to control pollen beetle (*Meligethes* spp.) are limited. Although effective organic insecticides are available (e.g. Spinosad), their use is often restricted by guidelines of producers associations. In Swiss organic production (Bio Suisse), as well as in Swiss IPM-production (IP-SUISSE) the use of insecticides in oilseed rape and cereals is restricted. Therefore, alternative non-insecticidal methods to control pollen beetles are needed.

Treatments with rock dusts for soil amendment, disease prevention or insect control have a long tradition in organic agriculture. However, little research was conducted to investigate efficacy and mode of action of different inert dusts (Ebling & Wagner, 1959, Ulrichs *et al.*, 2006, Wagner & Ebling, 1959). Only the use of amorphous silica (diatomaceous earth) for pest regulation in stored products is well studied (Golob, 1997). It was shown that diatomaceous earth does not kill by poisoning or suffocation, but by desiccation: the protective wax layer covering the insect's body is lost either by abrasion or absorption, leading to an increased water loss and consequently death of the insect (Ebling & Wagner, 1959, Ulrichs *et al.*, 2006). By comparing different abrasive and sorptive dusts, Ebling &

Wagner (1959) and Ulrichs *et al.* (2006) showed that sorptive dusts were more effective than abrasive dusts.

Another well-studied inert dust used for pest control is kaolin-clay (Glenn *et al.*, 1999). The particle film technology using kaolin was developed in the 1990's and is now used against different pest insects in perennial crops (Bürgel *et al.*, 2005, Daniel *et al.*, 2005, Saour & Makee, 2004). It is assumed that processed-kaolin particle film technology does not kill insects, but has a repellent and/or barrier effect: Plants may become unrecognisable (by affecting visual and/or tactile host-location cues), and feeding and oviposition may be impaired by the attachment of particles to the bodies of the insects as they crawl upon the film (Glenn *et al.*, 1999, Wyss & Daniel, 2004). References on other inert dusts for pest control are few (Humphrys & Jossi, 2009, Ulrichs *et al.*, 2006, Wagner & Ebling, 1959).

Clinoptilolite is a naturally occurring Zeolite: a microporous, aluminosilicate mineral commonly used as commercial adsorbent or deodorizer. In agriculture, Clinoptilolite is used as feed additive in animal production or as soil conditioner. No studies are available on the effects of Clinoptilolite for pest control. In this series of experiments the efficacy of Clinoptilolite to control pollen beetle (*Meligethes* spp.) was investigated.

## Material and methods

Experiments were conducted in Switzerland on commercial organic and IPM farms. Large-scale experiments were set up to monitor the migration of pollen beetle and compare between untreated plots and plots treated with the test materials using standard application equipment.

### *Experiments in 2008*

In 2008, the first pilot experiments were conducted in seven organic winter oilseed rape fields (total area: 4.2 ha) in North-western Switzerland. One half of each field was treated, the other half remained untreated. In the two largest fields, two replicates were set out (two treated and two untreated plots per field). Treated plots had an average size of 0.3 ha (0.15-0.40 ha).

Inert dust can either be sprayed or dusted onto the crop. Dust applications were conducted in six fields using a Vicon pendulum spreader, or a drop-box-fertiliser-spreader. The product Klinofeed (Clinoptilolite; Unipoint, Switzerland; particle size: 90% < 100 µm) was applied at rates of 300-750 kg/ha per application. Spray applications of Klinospray (Clinoptilolite; Unipoint, Switzerland; particle size: 17 µm; 50 kg/ha) in combination with a pinolene-based wetting agent (Heliosol; Omya AG Agro, Switzerland; 2 l/ha) were conducted in one field (two replicates) with a standard field sprayer using 600 l water/ha. The first applications were applied when buds were visible (stage 51 BBCH). Two or three treatments were applied during blossom development in order to compensate for washing off by rainfall. The last application was applied at growth stage 56-59.

### *Experiments in 2009*

In 2009, one experiment was conducted in an organic winter oilseed rape field (area: 3.8 ha) in the same region in North-western Switzerland as in 2008. Four replicates were set up in a block design. Treated plots were 0.48 ha. The product Klinofeed (300 kg/ha) was applied at growth stage 51 BBCH (15.04.2009) and at growth stage 58 BBCH (25.04.2009) using a drop-box-fertiliser-spreader.

### ***Experiments in 2010***

The experiments in 2010 were conducted in six IPM winter oilseed rape fields (total area: 9.0 ha) in North-western and South-western Switzerland. One or two replicates per field were set up (total: 7 replicates). Treated plots had an average size of 0.36 ha (0.17-0.6 ha). Because the application technique for dust applications was not available on IPM-farms, spray applications of Klinospray (30 kg/ha + wetting agent Heliosol 2 l/ha, with 600 l water/ha) were applied at growth stage 51-52 BBCH, 53 and 55-56 (only fields in North-western Switzerland with high infestation pressure).

### ***Sampling***

All sampling was done at five sampling points in the centre of each plot. At each sample point, five plants were selected at random and the number of adult pollen beetles per plant was assessed by beating the plant over a plastic tray. Assessments were conducted around noon (10:00-14:30 o'clock) from growth stage 50-60 BBCH. In Mid-June, plant density was determined by counting the number of plants per m<sup>2</sup> at each sampling point. Plant samples were taken (3 plants per sampling point) by cutting the plants at ground level. Assessments included fresh weight per plant, stem-diameter at ground level, plant height, number of side shoots, pods per main shoot, pods per plant, pods damaged by pod midge, number of podless stalks (damage by pollen beetles) per main shoot, length of galleries inside the stem (damage of stem mining insects). Fresh weight and pods per m<sup>2</sup> were calculated.

### ***Statistical analysis***

Data were analysed using the software JMP5.0.1. Data from 2008 and 2010 were tested for normal distribution and homogeneity of variances and analysed by two-way ANOVA [factors: treatment, field]. Data from 2009 were [ $\sqrt{x}$ ]-transformed to obtain normal distribution and homogeneity of variances and then analysed by one-way or two-way ANOVA [factors: treatment, block]. Data given in figures and text are means with standard deviations.

## **Results**

### ***Experiments in 2008***

Climatic conditions, plant development, and the start of pollen beetle immigration are given in Table 1.

Under the very wet and rainy conditions in 2008, only Klinofeed at the highest application rate (750 kg/ha) significantly reduced the number of beetles per plant one day after treatment ( $3.1 \pm 2.0$  beetles/plant) compared to the untreated control ( $6.5 \pm 3.0$  beetles/plant; efficacy: 48%). A lower application rate (350 kg/ha) resulted in  $6.0 \pm 2.3$  beetles/plant (efficacy: 8%; two-way ANOVA [application rate, field],  $F_{2,13} = 7.0$ ,  $p = 0.009$ , Tukey HSD-test). Three days after treatment, the effect of the highest application rate was no longer observed (two-way ANOVA [application rate, field],  $F_{2,13} = 2.7$ ,  $p = 0.1$ ).

Spray applications of Klinospray at a rate of 50 kg/ha did not reduce the number of beetles per plant one day after application (control:  $9.6 \pm 0.4$  beetles/plant; Klinospray:  $12.9 \pm 1.1$  beetles/plant).

Observations during sampling indicate that the beetles in the Klinofeed treated plots were covered in white powder and were visibly less active than beetles in the control plots. In the Klinospray treated plots, a similar reduction in activity was observed; however, it was less

pronounced. In the plots treated with Klinofeed, flowering started about 5 days earlier than in the control plots.

Five of the seven fields were in very bad condition due to the cold, rainy weather leading to limited nitrogen mineralization from the organic fertilizer applied. These plots were therefore ploughed-up during flowering. Only the two largest fields remained until harvest (one field treated with Klinospray, one field treated with Klinofeed; both with two replicates per field). The Number of pods per main shoot was increased by 83% after three applications with 750 kg Klinofeed and by 58% after three treatments with 50 kg Klinospray. The number of pods per plant was increased by 52% (Klinofeed) and 14% (Klinospray), respectively. The number of pods per m<sup>2</sup> was increased by 82% (Klinofeed) and 37% (Klinospray), respectively.

Table 1. Plant development, first observations of *Meligethes* spp. on winter oilseed rape plants and climatic conditions during April in the years 2008-2010

| Year   | 2008          | 2009          | 2010                                       |
|--|---------------|---------------|--|
| <i>Plant development</i>   |               |               |  |
| BBCH 51  | 05.04.-15.04. | 03.04.-08.04. | 07.04.-14.04.                              |
| BBCH 61  | 01.05.-05.05. | 21.04.-10.05. | 25.04.-30.04.                              |
| <i>First observations of Meligethes on oilseed rape plants and average number of Meligethes per plant at stage 51, 52-53, and 57-58 BBCH</i> |               |               |  |
| First observations   | 15.02.        | 03.04.        | 19.03. <sup>a</sup> (17.04. <sup>b</sup> ) |
| <i>Meligethes</i> / plant:   |               |               |  |
| stage 51 BBCH  | 2.6           | 0.8           | 3.4 <sup>a</sup> (0.2 <sup>b</sup> )       |
| stage 52-53 BBCH   | 5.3           | 4.6           | 15.5 <sup>a</sup> (2.9 <sup>b</sup> )      |
| stage 57-58 BBCH   | 8.3           | 2.6           | 11.2 <sup>a</sup> (3.4 <sup>b</sup> )      |
| <i>Climatic conditions during April</i>  |               |               |  |
| Mean Temperature °C  | 8.6           | 12.1          | 10.5                                       |
| Min. Temperature °C  | -3.2          | 2             | -0.7                                       |
| Max. Temperature °C  | 21            | 23.4          | 26.6                                       |
| Precipitation (mm)   | 202.2         | 19.6          | 32.4                                       |

<sup>a</sup>fields in north-western Switzerland 2010; <sup>b</sup>field in south-western Switzerland 2010 ;

### ***Experiments in 2009***

In 2009, Klinofeed (dust application, 300 kg/ha, BBCH 51-52) was applied under dry and sunny weather conditions. One day after treatment, the number of beetles per plant was significantly reduced by 73% (Figure 1). Nine (rainless) days after treatment at growth stage 58 BBCH, efficacy was 78% (Figure 1). Plant samples were taken after flowering to determine pod set. Damage by pollen beetles (podless stalks) was significantly reduced by 17% (Figure 1).

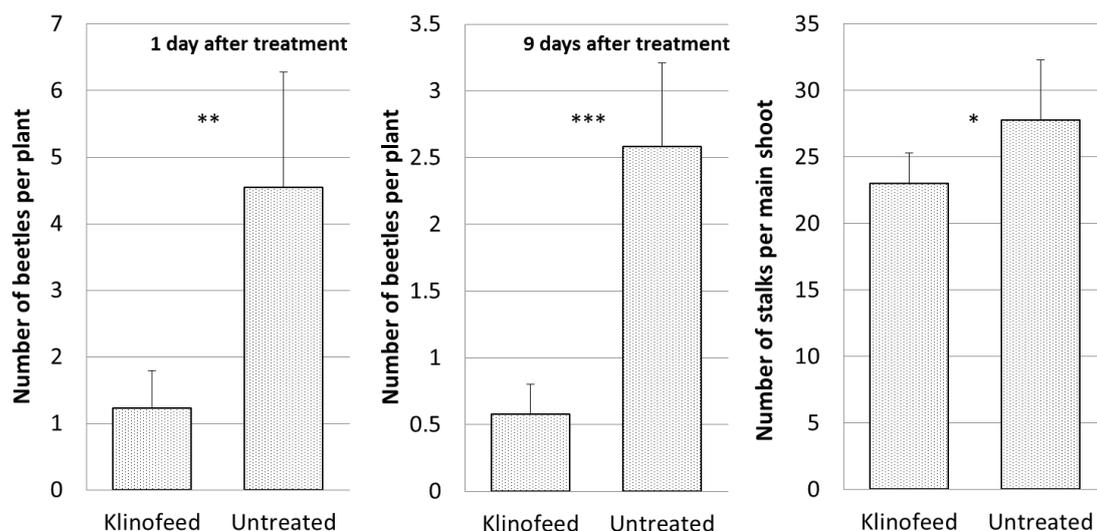


Figure 1. Effect of Klinofeed dust applications on number of pollen beetle one (BBCH 52) and nine days (BBCH 58) after application, and pollen beetle damage (podless stalks) per main shoot in 2009. Statistics: data transformed  $[\sqrt{(x)}]$ ; One day: one-way ANOVA,  $F_{1,6} = 17.8$   $p = 0.006$ ; Nine days: one-way ANOVA,  $F_{1,6} = 45.6$ ,  $p = 0.0005$ ; stalks: two-way ANOVA [treatment, block],  $F_{1,3} = 10.8$ ,  $p = 0.046$ .

Numbers of pods per main shoot and per plant were increased by 46% and 34%, respectively (Figure 2). The number of pods per  $m^2$  was significantly increased by 100% (Figure 2). However, no increase in yield was observed at harvest; treated plots yielded  $1130 \pm 130$  kg / ha, untreated plots yielded  $1150 \pm 260$  kg / ha. These results indicate that nitrogen availability instead of the pollen beetle damage was the yield-limiting factor under the organic agricultural conditions in 2009.

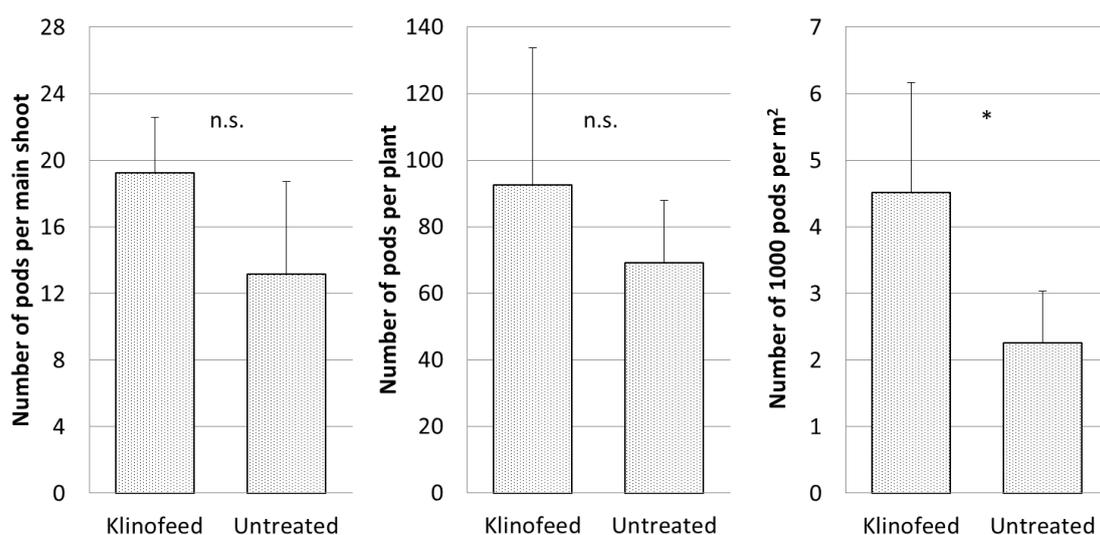


Figure 2. Effect of Klinofeed dust applications on the number of pods per main shoot, per plant and per  $m^2$  in 2009. Statistics: data transformed  $[\sqrt{(x)}]$ , Two-way ANOVA; main shoot:  $F_{1,3} = 8.07$ ,  $p = 0.067$ ; plant:  $F_{1,3} = 4.3$ ,  $p = 0.13$ ;  $m^2$ :  $F_{1,3} = 14.1$ ,  $p = 0.03$ .

### Experiments in 2010

Experiments in 2010 were conducted on IPM fields with a considerably higher nitrogen supply. In addition, infestation pressure with pollen beetle in 2010 was substantially higher than in 2009 (Table 1). Similar to the conditions in 2009, the weather remained sunny and almost rainless during the whole experimental period until start of flowering (growth stage 60 BBCH). Spray applications of 30 kg Klinospray/ha at growth stage 51 BBCH significantly reduced the number of beetles per plant and resulted in an efficacy of 52%, 45% and 50% one day, three days and seven days after the first application, respectively (Figure 3). Three days after the second treatment, efficacy was 22% (Figure 3). Five days after the last application (BBCH 59), 23% more pollen beetles were observed in the treated plots as compared to the untreated control (Figure 3). This observation might be due to the near-complete damage of the untreated plots: beetles might have left the control plots in search of flowering plants.

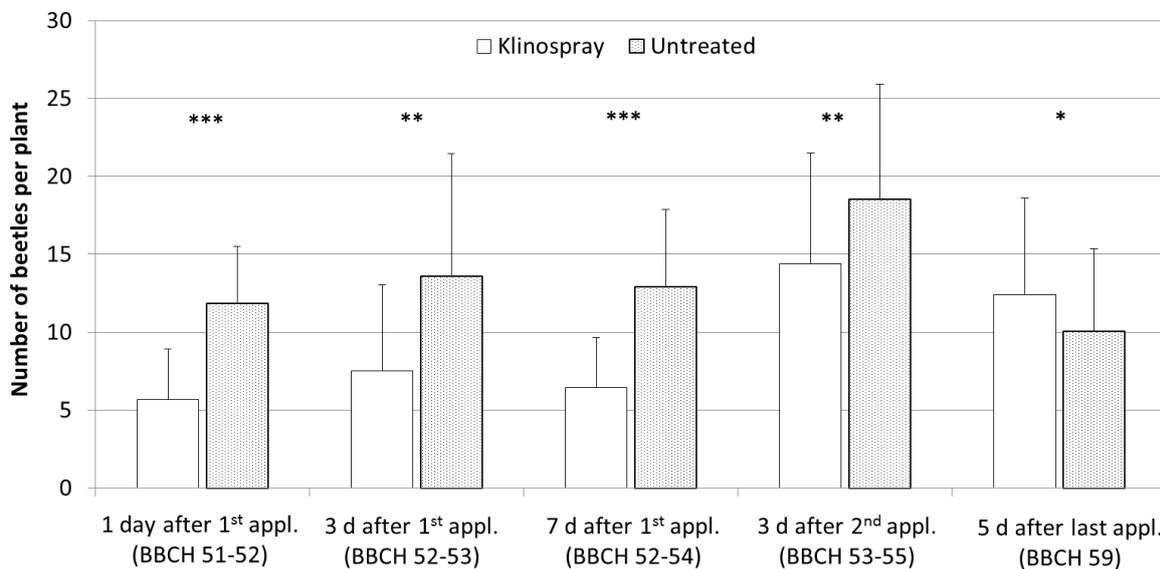


Figure 3. Effect of Klinospray applications on the number of pollen beetles per plant in 2010. Statistics: two-way ANOVA [treatment, field]; BBCH 51-52:  $F_{1,5} = 53.2$ ,  $p = 0.0008$ ; BBCH 52-53:  $F_{1,6} = 23.5$ ,  $p = 0.003$ ; BBCH 52-54:  $F_{1,6} = 52.9$ ,  $p = 0.0008$ ; BBCH 53-55:  $F_{1,6} = 19.1$ ,  $p = 0.005$ ; BBCH 59:  $F_{1,6} = 6.8$ ,  $p = 0.04$ .

Infestation levels differed substantially between experimental fields leading to large standard deviations (Figure 3). In south-western Switzerland, the number of beetles per plant remained below 5 for the whole experimental period, whereas in north-western Switzerland an average of 25 beetles per plant was observed at growth stage 54 BBCH. Nevertheless, efficacy was similar in all experimental fields.

The number of pods per main shoot was significantly increased by 53% in the Klinospray treated plots. The number of pods per plant and per  $m^2$ , however, were only increased by 9% and 11%, respectively (Figure 4).

Yield at harvest was significantly increased by 23% (two-way ANOVA, one field excluded because of heavy boar damage;  $F_{1,4} = 8.4$ ,  $p = 0.04$ ). Treated plots yielded  $1380 \pm 1260$  kg/ha, untreated plots yielded  $1120 \pm 1170$  kg/ha. Yield varied greatly between the different regions: with the low beetle pressure in South-western Switzerland, a yield of 3170 kg/ha was obtained from the untreated controls. Treatments with Klinospray increased

the yield by 11% (3520 kg/ha). With the high beetle pressure in North-western Switzerland, yield was considerably lower (610 kg/ha in the untreated plots). Treatments resulted in a 37% yield increase (840 kg/ha).

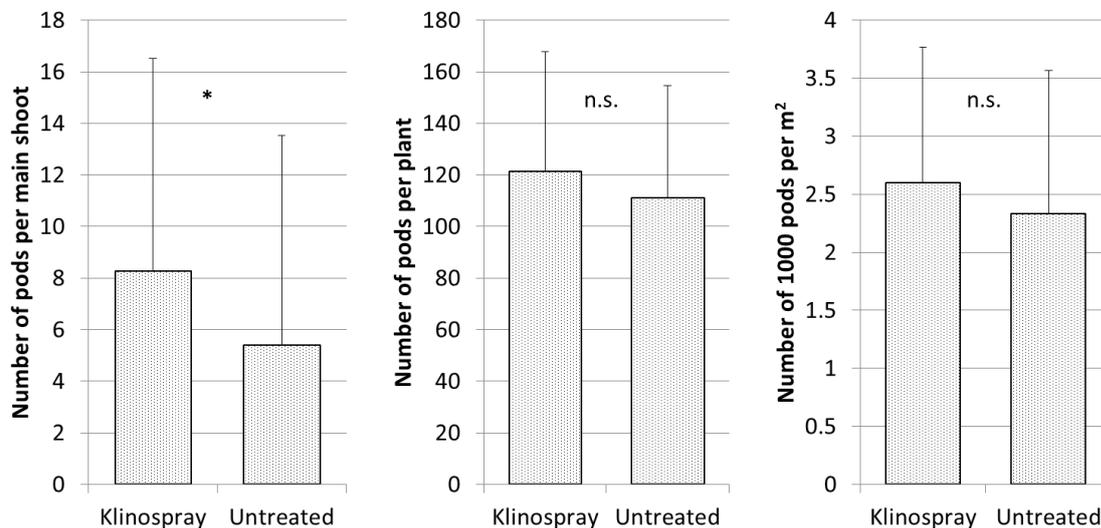


Figure 4. Effect of Klinospray applications on the number of pods per main shoot, per plant and per m<sup>2</sup> in 2010. Statistics: Two-way ANOVA [treatment, field]; main shoot:  $F_{1,6} = 6.3$ ,  $p = 0.04$ ; plant:  $F_{1,6} = 0.9$ ,  $p = 0.4$ ; m<sup>2</sup>:  $F_{1,6} = 3.9$ ,  $p = 0.09$ .

## Discussion

Efficacy of Clinoptilolite against pollen beetles ranged between 50 and 80% under dry weather conditions. Under the rainless conditions of 2009, Clinoptilolite provided good control (75% efficacy) throughout the crucial period of blossom development (growth stage 52-58 BBCH). No reduction of pollen beetles was observed under rainy weather conditions. Infestation levels differed substantially between experimental fields and years leading to large standard deviations. Nevertheless, efficacy was similar under high and low infestation pressure.

Efficacy during early stages of blossom development (BBCH 51-52) seemed to be higher than after elongation of the main inflorescence. This observation might be due to two reasons: Coverage of buds with Clinoptilolite and therefore exposure of pollen beetles to Clinoptilolite was much higher after an application at stage 51 BBCH than after an application at stage 55 BBCH. In addition Clinoptilolite, which is used as a deodorizer in cattle production, might have masked the typical odour of the oilseed rape plants and thus disrupt olfactory cues used to locate oilseed rape fields by migrating pollen beetles. This possible mode of action should be further investigated in olfactometer experiments. Moreover, Ebling (1971) showed that inert dusts also have a repellent effect on insects.

The mode of action of Clinoptilolite against pollen beetle is not absolutely clear. Observations of a higher efficacy under dry weather conditions indicate that Clinoptilolite – similar to other sorptive dusts (Mucha-Pezer *et al.*, 2008) might lead to a destruction of the protective wax layer and thus death of the insect by desiccation. Ebling (1971) assumes that desiccation of insects after removal of the protective wax layer is slower at higher relative

humidity and that insects can replenish their water loss in moist conditions. However, pore diameter in Clinoptilolite of 4 Å is considered to be too small for absorption of wax molecules. According to Ebling (1971) a pore diameter of at least 20 Å is necessary to absorb wax. In addition Ulrichs *et al.* (2006) showed in laboratory experiments on *Sitophilus granarius*, that mode of action of another zeolite product (Y-Zeolite) is not by desiccation.

In our experiments, dust applications seem to result in a higher efficacy than spray applications. This might be due to two reasons: (1) the lower application rates used in spray applications or (2) the saturation of the product with water and oil from the wetting agent. This again indicates wax absorption as possible mode of action; Ebling & Wagner (1959) showed that spray applications of sorptive dusts had a lower efficacy than dust applications.

The observation that pollen beetles in the Klinofeed treated plots were visibly less active than beetles in the control plots is in accordance with Ebling & Wagner (1959), who noted that treated insects were not as active as the untreated ones, indicating that the presence of dust may have adverse effects aside from that resulting from desiccation.

Three treatments of Klinospray at a rate of 30 kg/ha will cost 375 Euro (Klinospray: 50 Euro/25 kg; Heliosol: 15 Euro/l; labour and machine costs: 35 Euro/application). Thus, to be economically feasible, a yield increase of 230 kg/ha (in organic production; Swiss farm gate prize for organic oilseed rape: 16.5 Euro/t) and 470 kg/ha (in IPM production: farm gate prize: 8.0 Euro/t) would be necessary. The experiments showed that under IPM-conditions a yield increase of 230 to 350 kg/ha is possible. However, yields of 840 kg/ha, as obtained after treatments under severe pollen beetle pressure in 2010, are still far below farmer's expectations. No yield increase was observed under organic conditions.

## Acknowledgements

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# **Biocontrol**



## Insect pests and predators in oilseed rape relative to landscape and site factors

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**Abstract:** In thirty oilseed rape (OSR) fields located in landscapes ranging from structurally poor to complex in an agriculturally dominated region in Eastern Austria, abundance and diversity of OSR pests and arthropod predators were investigated at eight spatial scales. Abundance of pollen beetles was significantly negatively correlated with OSR area. Agrobiont spider species richness revealed the strongest positive response to amount of fallows at intermediate scales. Spider density was positively related to length of road-side strips with maximum effects at large scales. Nutritional condition of both sexes of the common carabids *Amara similata* and *Poecilus cupreus* increased significantly with OSR pest abundance. Number and biomass of spiders and carabids were significantly negatively correlated with the density of pollen beetles and stem weevils, indicating that these arthropod predators may prey upon these particularly severe pests of OSR.

**Key words:** carabids, fallows, nutritional condition, pollen beetle, spatial scales, spiders

### Introduction

The importance of oilseed rape (OSR) as a source for industrial and nutritional oil has been increasing worldwide and in some countries an increasing acreage is accompanied by a dramatic disproportionate increase of pesticide applications. Although OSR is able to compensate after insect damage, yield losses up to 80% have been reported when insecticide spraying was ceased (Hansen, 2003).

The present paper gives a preliminary resume of a recent 3-year project investigating insect pests, insect damage on OSR, and the numbers of potential epigeic antagonists (in particular spiders and carabid beetles) of OSR pests relative to landscape and site factors. The OSR pests studied were pollen beetles (*Meligethes aeneus*), stem weevils (*Ceutorhynchus pallidactylus*) and brassica pod midge (*Dasineura brassicae*), which were the most severe pests in the study region. Previously, only a few studies integrated both landscape and site factors, and even fewer have tried to estimate the actual spatial scale of the landscape impact (Schmidt & Tschardtke, 2005). So, this is, to our knowledge, the first study on insect pests and predators in arable crops that examines the effects of landscape and site factors at several spatial scales.

## Material and methods

Stem weevil larvae and adult pollen beetles were sampled in late April 2005 on 25 randomly chosen OSR plants. Brassica pod midge larvae were assessed in late May 2005 in 100 randomly chosen pods from the top racemes of OSR plants. All pests and their respective damage were surveyed along a 50 m transect located in the central area of each OSR study field.

Epigeic spiders and carabids were sampled using three pitfall traps, which were 20 m apart, per study field. Traps were emptied between the end of March and OSR harvest at the end of June 2005.

Effects of landscape and field factors on pest and predator numbers were analysed using ordinary least-squares regressions.

## Results and discussion

Pollen beetle density was significantly negatively correlated with amount of OSR area at both small and large spatial scales. Also, damage by pollen beetle and pod midge larvae were negatively related to OSR area, revealing that the amount of damage is correlated with pest density. Stem weevil damage increased with soil quality, which may have increased nutritional quality making OSR plants more attractive for stem weevils (Zaller *et al.*, 2008).

Total number of spider species revealed the strongest positive response to proportions of woody areas at small scales, and abundant agrobiont spider species were best explained by increasing proportion of fallows at intermediate scales (Figure 1). Spider density was positively related to the length of road-side strips with maximum effects at large scales.

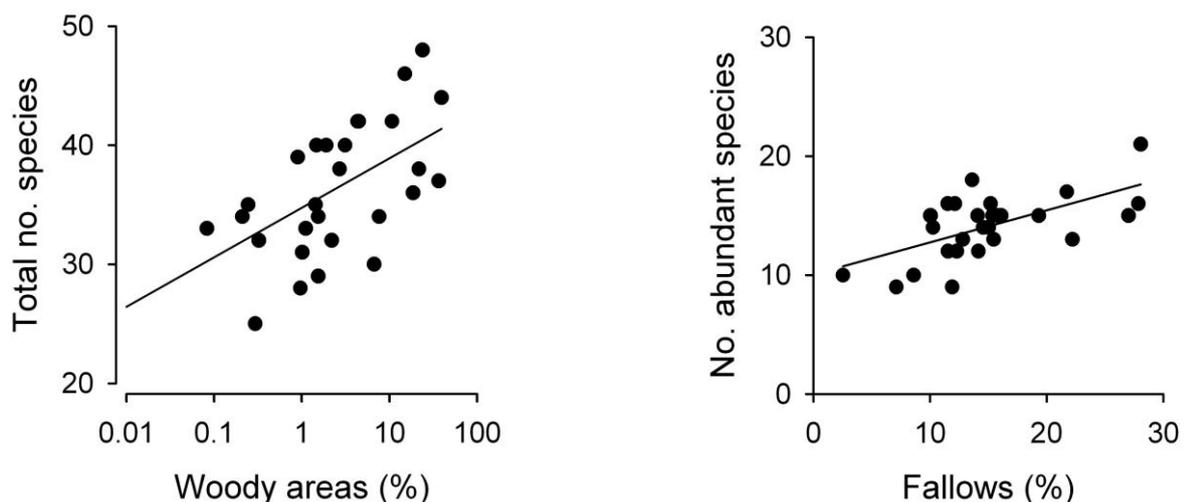


Figure 1. Relationships between total number of spider species and woody areas at a spatial scale of  $r = 500$  m, and number of abundant spider species and fallows at  $r = 1250$  m.

Similarly, activity-density of *Pardosa* spp., by far the most abundant spiders observed, was positively related to the length of road-side strips at radius 1750 m, combined with the smallest distance to the nearest fallow. Thus, these two types of semi-natural habitats were shown to increase spider density in OSR fields. Spiders did not respond to any field factors.

Nutritional condition of both sexes of the common predatory carabids *Amara similata* and *Poecilus cupreus* increased significantly with OSR pest abundance, and activity-density of *P. cupreus* and male *A. similata* decreased significantly with OSR pest abundance. High pest densities led to less active and satiated beetles. Oocyte numbers in the ovaries of female *A. similata* decreased significantly with distance to the nearest fallow, indicating that food available in fallows close to OSR fields contributes to enhanced reproduction. Number and biomass of spiders and carabids were significantly negatively correlated with the density of pollen beetles and stem weevils, indicating that these arthropod predators may prey upon these particularly severe pests of OSR therefore contributing to the reduction of pest numbers in OSR fields.

### **Outlook**

Current EU agro-policy abolished subsidies for set-aside land in 2008, which will cause an enormous loss of biodiversity in European agroecosystems (Tscharntke *et al.*, 2011). Therefore, it is necessary to observe what will happen to OSR pests and their antagonists in European OSR fields. To do this, we need scientific data on the interaction between OSR pests and their antagonists by comparing datasets surveyed before the EU set-aside abolition in 2008 with those after the abolition. Such knowledge is urgently required in the near future in order to better understand the impact of the rapid disappearance of set-aside land on OSR yield, OSR infestation by pests, and species richness as well as on numbers and efficacy of pest antagonists.

### **Acknowledgements**

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## **Winter activity of predaceous larvae of Cantharidae (Coleoptera) in oilseed rape crop**

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**Abstract:** Larvae of the soldier beetle (Coleoptera: Cantharidae) are predators, able to gain the food they need even at low temperatures. The aim of this study was to describe the impact of climatic factors on the activity of soldier beetle larvae in winter oilseed rape crops, during the vegetation period. Insects were caught by pitfall traps from October to the end of March in 2007-2009 in two crop fields differing in microclimate. The larvae of soldier beetles show activity from the early autumn until the end of winter, as long as the daily average temperature is not lower than minus 3.0 °C. Larval activity under snow cover was confirmed when the daily average minimum temperature reached minus 8.0 °C. However, the larvae had greatest activity at the end of October, when the average temperatures oscillate in between 3.8-6.0 °C.

**Key words:** Cantharidae, larvae, *Brassica napus*, winter activity, predators

## **News flash: Predator biomass not diversity drives natural biological control**

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**Abstract:** Pest suppression by predators is well documented as an important ecosystem service. The aim of this research was to investigate the role of carabid predator diversity and biomass in the suppression of pollen beetle larvae, a pest of winter oilseed rape. This was achieved by examining the individual performances of predator species (identity effect) and interactions among species (diversity effect) at low and high levels of predator biomass (biomass effect). A simplex design was employed in a large scale laboratory manipulation experiment which allowed the use of monocultures and mixtures of predators at fixed levels of overall biomass. Pollen beetle larval survival rate declined significantly as carabid predator diversity increased – diversity effect. As predator biomass increased, larval survival rate decreased – biomass effect. The impact of increased predator biomass outweighed the positive effect of predator diversity. To contextualise the importance of natural biocontrol of insect pests the impact of pesticide regimes on winter oilseed rape crop yield, carabid predator richness and abundance was also examined. There was no significant difference between high and low pesticide application and crop yield. However, pesticide application had a significant effect on predator biomass, with a reduction in carabid biomass in crops with high pesticide application. Pesticide management practices are having a detrimental effect on a natural biocontrol service nature freely provides, while in parallel man is incurring agri-economic cost and yet failing to achieve a higher crop yield. Here we show that carabid predator biomass drives the ecosystem service of natural biocontrol. Agricultural management practices, such as integrated pest control, will enhance carabid biomass and diversity therein helping to conserve and promote natural biocontrol.

**Key words:** carabid predators; pollen beetle; larval survival rate; pesticide side effects

## Using molecular methods to measure predation of oilseed rape pests

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**Abstract:** Pollen beetles, *Meligethes aeneus* (Fabricius) (Coleoptera: Nitidulidae), and flea beetles, *Phyllotreta* spp. (Coleoptera: Chrysomelidae) are major pests in oilseed rape, *Brassica napus* L. (Brassicaceae). Among the predator species in the generalist predator complex present in oilseed rape fields, wolf spiders (Araneae: Lycosidae) are found on the ground and cobweb spiders (Araneae: Theridiidae) build webs in the foliage. Here we study the incidence of predation of pollen beetles and flea beetles by these two spider groups using DNA-based molecular analysis. Wolf spiders of the genus *Pardosa* and the cobweb spider, *Theridion impressum* L. Koch, were each collected in six oilseed rape (OSR) fields (three winter OSR and three spring OSR). Pollen beetle and flea beetle densities as well as the occurrence of predators and alternative prey were monitored. In winter oilseed rape fields 13.8% of the collected *Pardosa* tested positive for pollen beetle DNA in the PCR analyses whereas 51.7% *T. impressum* were positive. The likelihood of detecting pollen beetle DNA in the gut contents of both spider groups was positively related to pollen beetle larval density. In spring oilseed rape fields 67.1% *T. impressum* tested positive for pollen beetle DNA, but less than 10% were positive for flea beetle DNA (both for winter and spring OSR). The implications of these results for conservation biological control and future studies of food webs in oilseed rape are discussed.

**Key words:** Pollen beetles, *Meligethes aeneus*, flea beetles, *Phyllotreta* spp., predators, predation spiders, PCR, gut content

## How to reinforce pollen beetle biocontrol at landscape level using a spatially explicit model

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**Abstract:** The intensification of agriculture has led to a loss of biodiversity, and subsequently a decrease in ecosystem services, such as natural regulation. Winter Oilseed Rape is an important oil crop in Europe and has to cope with numerous insects, which induce high pesticide use. Among the damaging insects, pollen beetle is the target of numerous pesticide utilizations, while natural regulation is known to induce high mortality rates. It appears that assessment of biological regulation of pests requires a landscape perspective (Bianchi *et al.*, 2010) and modelling is now considered a powerful tool to infer mechanisms from spatial patterns (Vinatier *et al.*, 2011b), and especially for tri-trophic interactions between plant, pest, and parasitoids. Therefore, we developed a lattice model called Mosaic-Pest to simulate the spatio-temporal dynamics of cohorts of pollen beetles and parasitoids in relation with landscape composition and structure. The model describes the most important processes (dispersal, mortality and fecundity) affecting population structure in space and time. Crop allocation in space and time, ploughing, and use of trap crops were explicitly considered in the model, on the basis of their influence on beetle and parasitoid populations. Species-specific parameters were derived from the literature available on the species or its closed taxon. Landscape mosaic and especially semi-natural habitats and oilseed rape crops were defined on the basis of GIS maps collected in north-western France, figuring contrasting situations in terms of landscape complexity. We tested various combinations of cultural practices affecting population of pollen beetles to select innovative integrated pest management strategies. The model showed a negative effect of trap crop on pollen beetle densities due to a diminution of pollen beetle egg-laying and an enhancement of parasitism. The model showed a relative low effect of ploughed soil tillage compared to other cultural practices but with a negative effect on pollen beetles infestations. Interaction between crop rotation and biological regulation was negative, because long crop rotation decreased the connectivity between fields, and consequently increased the mortality rate of both pollen beetles and parasitoids during dispersal events.

**Key words:** lattice model, Pollen beetles, natural regulations, cultural practices, parasitoid

### Introduction

Biological regulation of pests is a complex process affected by both landscape configuration and agricultural practices. There is an urgent need to develop modeling tools to help design innovative Integrated Pest Management (IPM) strategies that consider tri-trophic interactions at landscape scales. However, there is still a lack of landscape models that consider agricultural practices as levers to enhance biological regulation. To this end, we developed a grid-based lattice model called Mosaic-Pest and we took as case studies the pollen beetle (*Meligethes aeneus* F.) (Coleoptera, Nitidulidae) and its parasitoid *Tersilochus heterocerus*.

The pollen beetle is a major pest of winter oilseed rape (*Brassica napus* L.), because adults feed on pollen and oviposit on buds causing major bud abortions. Pollen beetles can be regulated by univoltine parasitoids, especially *Tersilochus heterocerus* (Rusch *et al.*, 2010; 2011). Both the parasitoid and its host require complementary and supplementary resources

for feeding, egg-laying, and overwintering. Resources are semi-natural habitats, i.e. woodlands and grasslands, and cultural areas with their associated practices. Recent studies pointed out the contrary effects of some landscape elements in pest regulation, because semi-natural habitats act both as overwintering sites for pollen beetle and as nectar sources for parasitoids (Rusch *et al.*, 2012). Agricultural practices such as ploughing seem to affect pest regulation at landscape scale because mouldboard ploughing reduces parasitoid survival and emergence (Nilsson, 2010). A push-pull strategy takes advantage of the preference of *M. aeneus* for turnip rape (*Brassica rapa*) over oilseed rape, to use it as a trap crop for pollen beetle (Cook *et al.*, 2007). However, the link between mechanisms and observed patterns at the landscape scale remains poorly understood, in particular because spatio-temporal dynamics of the landscape in previous years has spillover effects on pest dynamics. It is also necessary to consider both positive and negative effects of semi-natural habitats and of cultural practices with a modelling approach.

## Material and methods

### *Spatial representation of landscapes in the model*

We chose a spatially explicit framework taking into account four different habitat types: oilseed rape fields, previous oilseed rape fields, woodlands, and grasslands, because of their different influence on insect overwintering, feeding, and egg-laying. The rest of the grid consists of unsuitable habitats. Landscape maps were projected onto a grid of  $100 \times 100$  cells with a cell length of 50 m. Border effects in regard to dispersal were avoided by a toroidal structure of the landscape.

### *Cultural practices used to test the model*

Delimitation of each field was identified from aerial photographs (BD ORTHO<sup>®</sup>, IGN, 2004) and completed by the official SIG-based system used by farmers to declare crops and apply for subsidies. A unique identification number is assigned to each cell belonging to a given field. Presence or absence of ploughing was assessed simultaneously on all the fields of the map. When we considered the effect of trap crop, we assigned turnip rape for each cell at the internal border of contiguous fields containing oilseed rape. Crop allocation in space and time were determined by the duration (in years) of the crop rotation sequence. At the beginning of the simulation, we assigned randomly a crop sequence position number to each field.

## Results and discussion

### *Model description*

We assigned to every cell of the grid a number of parasitoids or pollen beetles, at a given stage for immatures, or status for adults. Mosaic-Pest progresses with daily time steps. Every simulation begins the 1st of January. At this date, parasitoids and pollen beetle are overwintering in the soil of oilseed rape fields of the previous year and woodlands, respectively. We considered the initial number of insects per cell to be the same for all cells of a particular type in all maps. Populations of parasitoids change stages from immature to adult, and populations of *M. aeneus* from egg, 1st instar larvae, 2nd instar larvae, pupa to adult. Adults also change status during their life cycle until death, from overwintering, emerging, dispersing for feeding, dispersing for egg-laying, to egg-laying. The new generation of *T. heterocerus* stays as diapausing adults within the soil, whereas new adults of *M. aeneus*

disperse to the overwintering sites, i.e. woodlands. Oilseed rape plants pass through three different stages until harvest, from preflowering, flowering to postflowering (Figure 1).

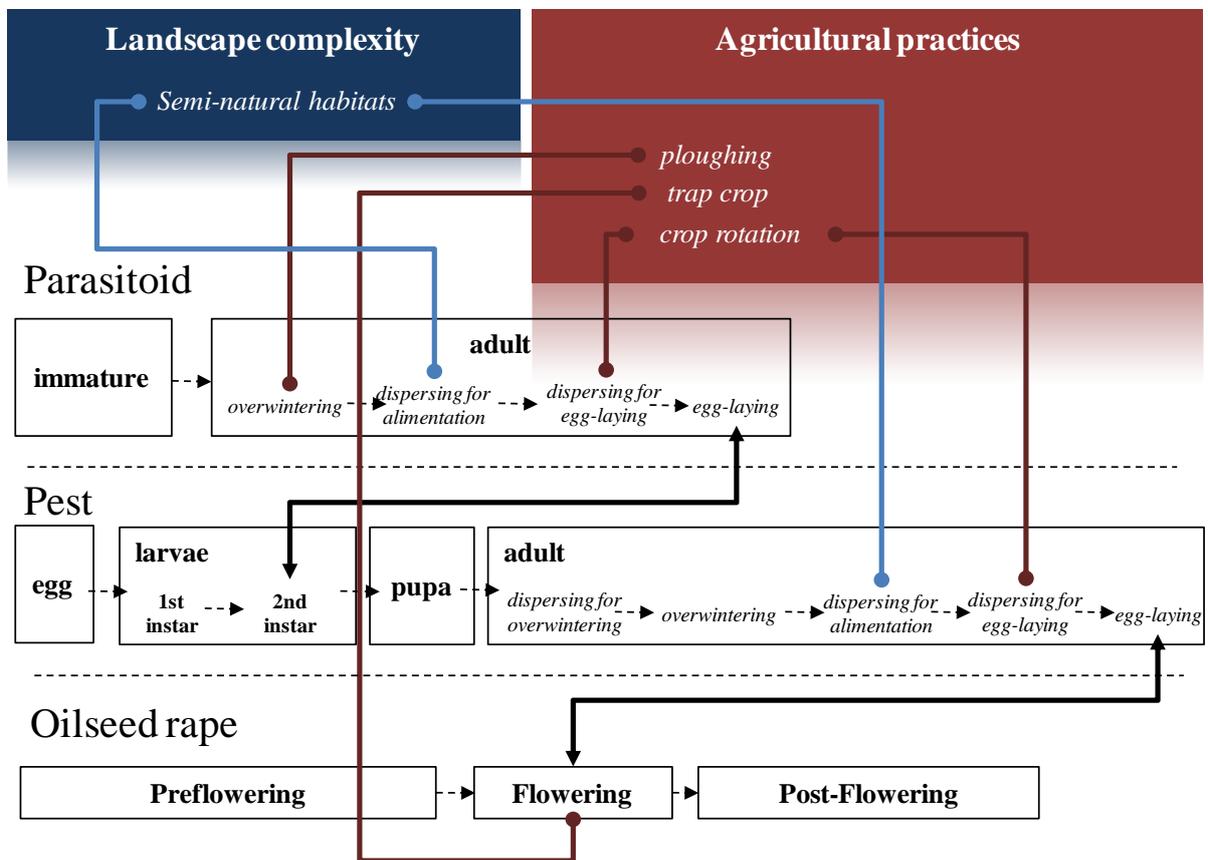


Figure 1. Overview of stages (in bold) and status (in italic) decomposition of each level of the tri-trophic system. Bold arrows correspond to interactions between levels. Dotted arrows correspond to transition rates between each status.

### *Spatial processes*

Dispersal occurs just after emergence of the species. Two events of dispersion occur successively: to oilseed rape fields and grasslands (feeding sites) and then to oilseed rape fields (egg-laying sites). We considered a third event only for *M. aeneus*, as the next generation disperses to overwintering sites, i.e. woodlands.

We consider a cell to cell redistribution mechanism based on a markov chain (Vinatier *et al.*, 2011a). For each cell of the grid, the population is redistributed according to the potentials of the cells of its perception window. Each cell's potential depends on (i) the dispersal abilities of the species and its physiological state, and (ii) the habitat attractiveness that depends on the species and the status of their population. For both *M. aeneus* and *T. heterocerus* at the end of the overwintering period, we considered that grasslands and oilseed rape fields are equally attractive for feeding, and all other habitats are non-attractive. After feeding, we considered that insects fly exclusively to oilseed rape fields, and we set the relative preference of other habitats to 0. Effect of trap crops consisting of turnip rape was added in the model by setting  $\alpha_{\text{turnip rape}} = 17 \times \alpha_{\text{oilseed rape}}$  fields.

### ***Temporal processes***

For each species, at the end of a given stage/status, determined by the developmental time, the population number of the cohort is multiplied by a transition rate that depends on species traits. The survival rate is defined by a constant for immature stages of the species and depends on the ploughing activity for *T. heterocerus*. For adults, the survival rate depends on the power of the distance covered by the population during dispersal. The total collapse of a cohort is determined by the maximal longevity of the species.

Egg-laying by adult *M. aeneus* occurs from the beginning to the end of oilseed rape flowering period, with a maximal duration of 60 days. The mean number of eggs per adult per day is considered constant. During the parasitism phase, the number of parasitized hosts per cell is given by the Thompson model with type II functional response.

### ***Effects of cultural practices during long-term simulations***

The duration of each simulation was 10 years for studying long term effects on the population. At the end of each year, we collected the mean number per m<sup>2</sup> of pollen beetles adults and the parasitism rates and we average the value over the 10 repetitions. We tested effects of trap crop, ploughing, and crop rotation across the 35 map patterns and with or without biological regulation. The results of those simulations were analyzed using an ANOVA for pollen beetle densities and parasitism rates. Almost all variability was explained by this analysis either for pollen beetle densities ( $R^2 = 0.95$ ) or for parasitism rates ( $R^2 = 0.93$ ). Sensitivity indexes and signs of  $\beta$  coefficients indicated that *biological regulation* and *trap crop* had a strong negative and positive influence on pollen beetle densities, respectively. *Crop rotation* had a slightest influence on pollen beetle densities. On the other hand, impact of *ploughing* and *crop allocation* was negligible. The most important and positive interaction effect was for *crop rotation*  $\times$  *biological regulation*; despite that *crop rotation* itself had a weak influence on pollen beetle densities. Parasitism rates were strongly negatively influenced by *crop rotation*, then positively by *trap crop* in a slightest importance. Impact of *ploughing* and *crop allocation* was less important. The most important interaction effect was found for *crop rotation*  $\times$  *trap crop*. The negligible effect of ploughing on both pollen beetles densities and parasitism rates was surprising because it had been identified as significant by Rusch *et al.* (2011). The high impact of other cultural control may have hidden the effect of ploughed soil tillage. Furthermore, this weak influence of ploughing is overwhelmed by influences of crop rotation and trap crops, these cultural practices being not considered in the study of Rusch *et al.* (2011).

The ANOVA indicated that landscape configuration had a high effect on pollen beetle densities and parasitism rates. Landscape influence was almost of the same magnitude as some agricultural practices, such as trap crops, and biological regulation.

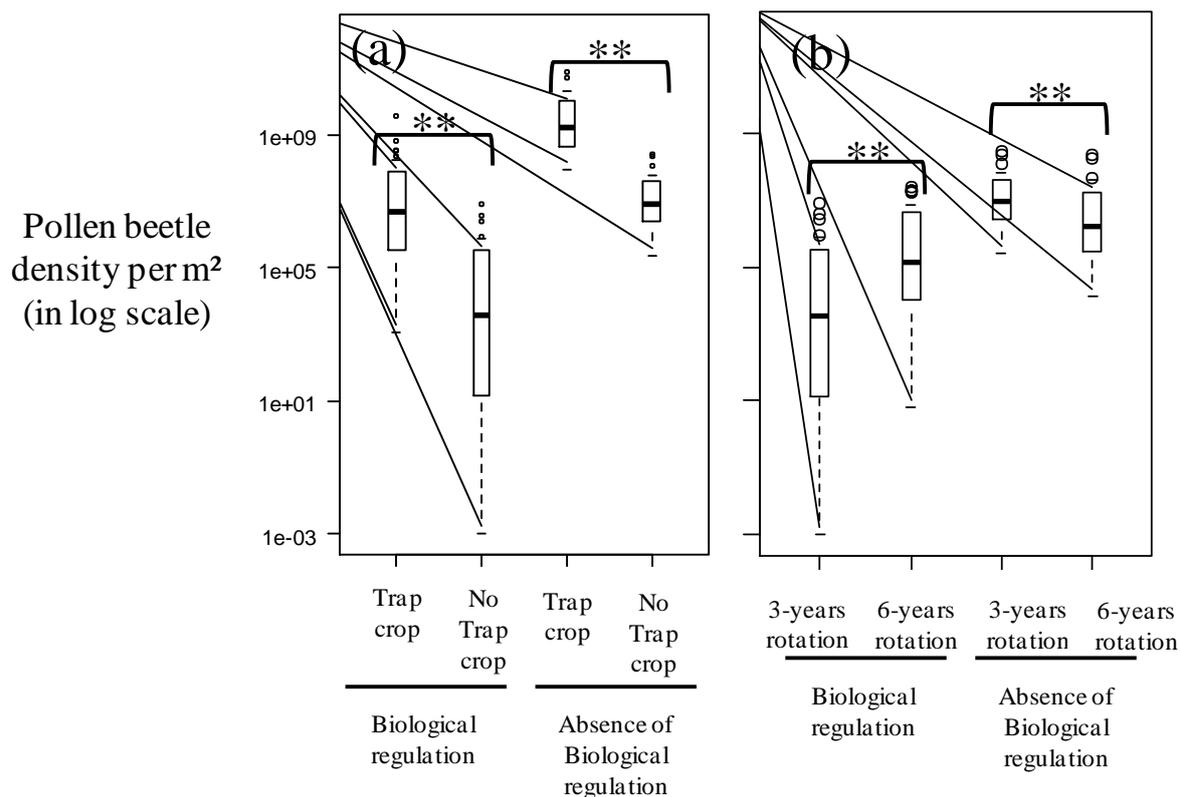


Figure 2. Boxplots ( $n = 35$ ) illustrating the ranges and median (horizontal black line) for pollen beetle density issued from a 10-years simulation using Mosaic-Pest and averaged over 10 repetitions for each map. We the ploughing as absent for all comparisons. Crop rotation was fixed to 3 years in (a) and trap crop were not considered in (b). Stars indicate a significant difference ( $P < 0.001$ , Mann-Whitney U test).

Figure 2 illustrated the interactions between cultural practices of importance and biological regulation. Large range of boxplots over the map patterns indicated the high influence of landscape composition and structure on the levels of insects. The negative effect of trap crop on pollen beetle densities (Figure 2a) was probably due to the periodicity of trap crop flowering in comparison with the oilseed rape that shortened the favorable period for pollen beetle egg-laying. This trap crop effect was emphasized by the concentration of pollen beetle densities due to trap crop attractiveness, and the enhancement of pollen beetle regulation, as shown by the positive influence of trap crop on parasitism rates. Figure 2b showed the significant effect of crop sequence duration. Interestingly, the interaction between crop rotation and biological regulation was negative. The model illustrated the negative effect of crop sequence duration on biological regulation, due to an increase in previous and current oilseed rape connectivity. Indeed, lengthening crop rotation decrease the connectivity of oilseed rape fields, i.e. distance between oilseed rape fields a given year. In absence of biological regulation, this had for consequence an increase of pollen beetles mortality during dispersal events and so a diminution of their densities. In presence of biological regulation, this mortality effect was counterbalanced by a limitation of biological regulation by parasitoids, the latter being also influenced by the connectivity between previous and current oilseed rape fields for a given year.

### ***The potential of Mosaic-Pest***

Mosaic-Pest helps understanding of complex systems such as tri-trophic interactions at the landscape scale. Researchers and stakeholders can test the influence of agricultural practices on pollen beetle regulation via Mosaic-Pest, the latter being considered as a “virtual laboratory” to select the best combination of practices that maximizes biological regulation.

Individual farmer practices could also be taken into account in the model, by adding a sub-model in Mosaic-Pest that considers agents (farmers) with their own decisions rules. Rather than considering cultural practices at landscape scale with the current version of Mosaic-pest, the new version can consider farmers decisions as levers to manipulate the system.

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## Accuracy of landscape indicators to predict levels of pollen beetle infestations and successful biological control in oilseed rape

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**Abstract:** Designing multifunctional landscapes requires accurate indicators to assess the impact of landscape structure on the provision of goods and services. Biological pest control relying on natural enemies is an important ecosystem service considered as a sustainable alternative to chemical control. The aim of this study is to measure and compare the accuracy of landscape indicators computed at various spatial scales to predict pollen beetle infestations and successful biological control in northwestern France. The sensitivity, specificity, and probability of correctly ranking fields were estimated for each indicator based on a survey of 42 fields using the receiver operating characteristic procedure. For pest infestation, the proportion of woodland and the proportion of semi-natural habitats were found to be informative indicators with good discriminatory abilities. For biological control, the proportion of woodland, the proportion of semi-natural habitats and the proportion of the previous year's oilseed rape fields with reduced soil tillage were found to be informative indicators with good discriminatory abilities. By using indicator values, optimal thresholds, and posterior probabilities, we were able to compute maps of areas at risk for pest infestation and those displaying successful biological control at the regional scale. This study provides tools that could help extension services, landscape planners, and policy makers in optimizing landscape structure according to the provision of a key ecosystem service. The results of this study also provide new grounds for understanding trophic interactions at the regional scale as well as the ambivalent effect of landscape complexity on pest and natural enemy populations.

**Key words:** *Meligethes aeneus*; risk assessment; landscape structure; spatial scales; woodlands; semi-natural habitats; reduced tillage

### Introduction

Concerns about the detrimental impacts of agrochemical inputs on the environment and their inevitable negative side-effects on non-target organisms have been growing since the 1960's. Therefore, developing ecologically-sound pest management strategies has become a major and urgent issue. Winter oilseed rape (*Brassica napus* L. - WOSR) has to cope with numerous damaging insects, resulting in the large-scale use of broad-spectrum insecticides; the largest component of the Treatment Frequency Index (TFI). Among insects, the pollen beetle (*Meligethes aeneus* Fabricius) (Coleoptera: Nitidulidae) is one of the most damaging pests of WOSR as its damage results in bud abortion (Alford *et al.*, 2003). The main method to control pollen beetle is the intensive use of insecticide treatments, but during the past decade, insecticide-resistance in pollen beetle populations has been an increasing phenomenon, spreading throughout most European countries (Detourne *et al.*, 2008). Hence, the control of pollen beetle has become a major concern for farmers, highlighting the need to develop innovative pest management strategies. Recently, some scientists have suggested that functional biodiversity at the large agro-ecosystem scale could maintain important ecological services such as the biocontrol of pests, which could constitute a crucial way to sustainably manage pests (Médiène *et al.*, 2011). Because of the specific biology and behaviour of pests,

their populations can be affected by multiple factors operating at various scales (Ricklefs, 1987; Clough *et al.*, 2007, Rusch *et al.*, 2010). While at the field scale, the richness and abundance of insect species depends on local habitat characteristics which are largely determined by crop management, at larger scales the richness and abundance of local species are strongly affected by landscape context, especially for high dispersive organisms and multiple-habitat species. Studying the relative crop management and influence of landscape context at different spatial scales may help to develop alternative pest management strategies, but such approaches are still rare (Zaller *et al.*, 2008; Eilers and Klein, 2009, Rusch *et al.*, 2011, 2012; Frank *et al.*, this volume). Additionally, to our knowledge there is no study that aims to propose decision support tools to identify risk areas where natural pest control is insufficient. The aims of this study were: (1) to identify and rank the determinants (local and landscape) of biological control of pollen beetles (2) to produce and compare landscape indicators, which take into account either semi-natural habitats or cropping system (soil tillage and WOSR area), which can discriminate landscapes according to potential success of natural pest control and to map at large-scale landscapes according to the prediction of successful biological control.

## **Material and methods**

### ***Experimental design***

On 42 farmers' fields in Normandie (northwestern France; (49°05'N, 1°16' E; see map in Rusch, 2010), we explored the relative influence of crop management and landscape context on the pollen beetle and its parasitoids over a two-year period (harvest years 2008-2009). Fields were selected according to their landscape complexity, cultivars, proportion of WOSR area in the region and spatial independence (distance between fields > 4 km). The complexity gradient ranged from simple and open landscapes with less than 3% of semi-natural habitats, to more complex and closed landscapes with more than 58% of semi-natural habitats. On each field, all measurements were made in an untreated 1 ha area.

### ***Landscape indicators***

Based on aerial photographs (BD ORTHO<sup>®</sup>, IGN, 2004, pixel size: 0.5m) and intensive field inspections, we manually digitalized the land use around each field and quantified the total area of each habitat type using ArcGis software (Version 9.2, ESRI). Crop management, including sowing date, density and fertilisers were recorded on each studied field while the location of previous years' OSR crops, cultivar and soil tillage were identified using interviews conducted with farmers identified in the area of a 2000m radius. Landscape variables and crop management (soil tillage, pesticide use) on OSR were assessed in buffers of 2000m radius, as this distance was demonstrated by Rusch *et al.* (2011) as the relevant spatial scale for parasitism. We chose a few simple landscape variables: proportion of land use (based on all types of land use) and proximity index to the previous years' OSR crops.

### ***Pest measurements and parasitism rate***

Adult pollen beetle abundance in the fields was assessed on three dates by counting the number of beetles on 50 randomly chosen plants along a transect. Parasitism rates of pollen beetle larvae were assessed at the end of flowering from 150 larvae sampled on 30 plants randomly selected along a transect. Pollen beetle larvae were sampled at the end of flowering (GS around 65-69) in order to provide a good estimation of maximum parasitism rates by all

possible parasitoid species. The individuals of larval stage L2 were dissected under a microscope to check for parasitism and species identified using Osborne (1960).

### ***Multi-model inference method***

In order to determine the major effect of landscape and cultural practices on biological control, we used linear mixed-effects modelling, with year and region as random effects. For each variable (parasitism rate or pollen beetle abundance), we examined the effects of the predictors using the multimodel inference approach. This entails fitting all possible linear combinations of the explanatory variables by least squares and computing, for each combination, the Akaike Information Criterion (AIC) value, and the Akaike weight (Burnham & Anderson, 2002). For model  $i$  the Akaike weight represents the probability that, given a set of models,  $i$  would be the AIC-best model (Burnham & Anderson, 2002). Estimates of the relative importance of a predictor can be made by summing the Akaike weights across all models in the set where this variable occurs. The relative importance of a predictor is reflected by the sum of these weights. The larger the sum the more important the variable is (Burnham & Anderson, 2002). Using these sums, all the variables can be ranked according to their importance. By summing the Akaike weights normalised on the whole database, we are able to calculate the relative importance of this buffer which can be interpreted as the probability that models including crop management and landscape variables calculated at the given buffer would be selected as the AIC-best model. We included into the analyses predictors that were not highly correlated with each other. Data were explicitly tested for normality and homogeneity of variance using the Shapiro-Wilk statistic and Levene's tests, respectively. Pollen beetle abundance were  $\log(x+1)$  transformed and proportions of destroyed buds were  $\text{asin}(\sqrt{x})$  transformed to account for heteroscedasticity and deviation from normality.

### ***Receiver operating characteristic (ROC) curve analysis***

Receiver operating characteristic (ROC) analysis was used to evaluate the ability of each landscape indicator to discriminate fields with successful pest control or not, based on decision threshold values defined thereafter. For the variable parasitism rate, fields were divided into two groups depending on whether this variable (termed  $D$ ) exceeded a predefined threshold (termed  $D_{th}$ ) or was below that threshold. The three threshold values used in the present study to categorize fields were:  $D_{th1} = 70\%$ ,  $D_{th2} = 80\%$  and  $D_{th3} = 90\%$ . A ROC analysis was performed separately for each landscape indicator at each spatial scale and for each value of  $D_{th}$ . We used here the methodology proposed by Hughes *et al.* (1999) and Makowski *et al.* (2005). The values of each landscape indicator at each spatial scale were then calculated for each field in both groups. Each indicator value ( $I$ ) was compared to a decision threshold ( $I_{th}$ ). These results were used to calculate the true positive proportion (TPP; sensitivity) and the true negative proportion (TNP; specificity). TPP is defined as the number of fields with  $I > I_{th}$  in the group of fields with  $D > D_{th}$  divided by the number of fields in that group; and TNP as the number of fields with  $I \leq I_{th}$  in the group of fields with  $D \leq D_{th}$  divided by the number of fields in that group. ROC curves show the relationship between the true positive proportion (TPP; sensitivity) and the false positive proportion (FPP; 1-specificity) across all possible values of  $I_{th}$  and for a given value of  $D_{th}$ . For each indicator at each spatial scale, ROC curves were thus created by plotting TPP against FPP. ROC curves show the relationship between the true positive proportion (TPP; sensitivity) and the false positive proportion (FPP; 1-specificity) across all possible values of  $I_{th}$  and for a given value of  $D_{th}$ . For each indicator at each spatial scale, ROC curves were thus created by plotting TPP against FPP. To evaluate and compare the accuracy of each indicator, the area under the ROC

curve (AUC) of each indicator was calculated. The AUC of a model can be interpreted as the probability that the indicator values for two randomly selected fields of positive and negative events will be correctly ranked (Makowski *et al.*, 2009). In practice, it is necessary to know the best cut-off point of an indicator to use as an operational threshold for decision-making. The threshold that minimizes the difference between sensitivity and specificity (curves crossed in Figure 1) is thus defined as the optimal threshold (Makowski *et al.*, 2009). We thus calculated the optimal threshold for every decision thresholds of all informative indicators.

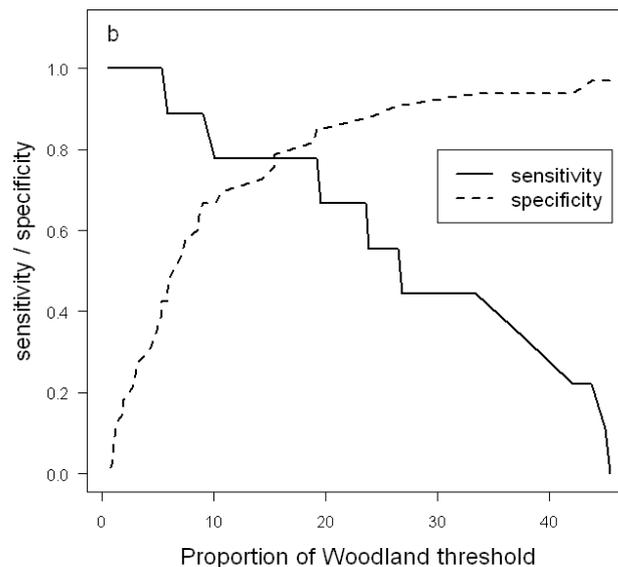


Figure 1. Sensitivity and specificity of the indicator ‘proportion of woodland’ in the 2000 m spatial scale for parasitism rates above  $D_{thp2}$  as a function of the indicator value.

### ***Optimal threshold and mapping***

In a reverse approach, for a given indicator  $I$  we calculated the posterior probabilities for all threshold values as defined in Makowski *et al.*, 2009. We computed these posterior probabilities for the indicator proportion of woodland at the 2000m scale. The probability of  $D > D_{th}$  for a new field before knowing the indicator value can be estimated from our dataset as the proportion of fields where  $D > D_{th}$  (Makowski *et al.*, 2009). All computations were performed using the statistical software R with the ROCR package. Based on the optimal thresholds identified for individual landscape indicators, we were able to produce binary maps with or without successful pest control over the entire region according to the indicator value. The value of each indicator at a given spatial scale was calculated for each point of the raster layer (cell size = 100 m x 100 m) using the CORINE Land Cover data base (France, IFEN, 2004) and ArcGis software (Version 9.2, ESRI), and were compared to the optimal threshold identified.

### ***Logistic regression***

At each spatial scale, different landscape indicators were combined to predict the probability of high pollen beetle infestation and successful natural pest control according to thresholds described previously, using logistic regression. Here, logistic regression was used to examine

the interest of using several indicators computed at the same spatial scale to predict the probability of high pest infestation or successful natural pest control for each decision threshold (Barbottin *et al.*, 2008). For pollen beetle infestation, we used the proportion of woodland, the proportion of grassland, the proportion of OSR and the Shannon index of habitat diversity. For parasitism rates, we combined the proportion of woodland, the proportion of grassland, the proportion of the previous year's OSR crops with reduced tillage and the Shannon index of habitat diversity. We used Akaike Information Criterion (AIC) and multimodel inference for parameters estimations and calculate the relative importance of each variable (Burnham and Anderson, 2002). For a set of indicators at a given spatial scale, multimodel inference approach considers all the possible models obtained from linear combinations of the indicators. Each model was then ranked according to its Akaike weight and parameter estimations which were computed by a weighted average of parameter estimates from models in which a given variable is explicitly present (Burnham and Anderson, 2002). As previously described, the discriminatory ability of each model was assessed using ROC curve analysis and AUC for each logistic regression at each scale. AUC values for logistic regressions were computed using leave-one-out cross validation to limit the risk of underestimation of the model.

## Results and discussion

### *Crop management and landscape context effects on parasitism rates*

Parasitism rates ranged from 0 to 98 %. We found a significant effect of year ( $F = 5.38$ ;  $P = 0.02$ ) and site ( $F = 6.83$ ;  $P = 0.01$ ) on total parasitism rates. In 2008, the mean rate of total parasitism was  $40.3\% \pm 27.1\%$  and in 2009 it was  $60.3\% \pm 28.9\%$ . Parasitism was mainly effected by three univoltine parasitoid species (*Tersilochus heterocerus*, *Phradis morionellus* and *interstitialis*) (accounting for more than 95% of total parasitism). The proportion of non-cropped areas greatly influenced parasitism rates in all buffers. Indeed, it always had a high probability to be selected in the best model in all buffers. At the 1500 m scale, the relative importance values of explanatory variables indicate that three predictors have a high probability of appearing in the best fitting model: the proportion of OSR<sub>n-1</sub> fields under conventional post-harvest soil tillage ( $w_+(j) = 0.62$ ), the proportion of woodland ( $w_+(j) = 0.73$ ) and the proportion of grassland ( $w_+(j) = 1.00$ ) (Figure 2b). These variables are thus important predictors for the total parasitism rate of pollen beetle larvae. In particular, the proportion of grassland at scales ranging from 1500m to 2000m was found to be the most important variable compared to other predictors for the total rate of parasitism (Figure 2). The estimated parameter values reveal that the proportion of grassland and the proportion of woodland have positive effects on parasitism rate. The proportion of OSR<sub>n-1</sub> fields under conventional post-harvest soil tillage in the 1500 m circular sector is negatively related to the rate of larval parasitism. Hence, OSR fields with high proportions of OSR<sub>n-1</sub> under reduced post-harvest soil tillage in the surrounding landscape seem to have a higher rate of parasitism than OSR fields with high proportions of OSR<sub>n-1</sub> fields under conventional tillage.

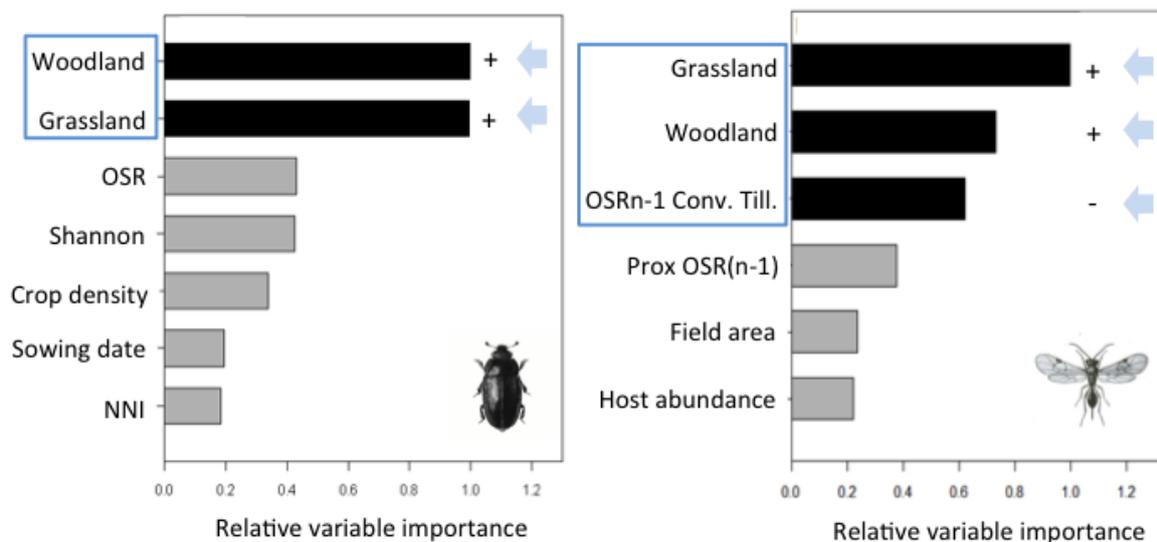


Figure 2. Relative importance of each variable in explaining variation in: Pollen beetle abundance (a) and total parasitism rates (b) with landscape predictors calculated at the 1500 m scale. Variables are ranked in order of the sum of their Akaike weights ( $w_+$  (j)), which are the probabilities that the given predictor would appear in the best fitting model (lowest AICc value). Black bars indicate the most important variables.

### ***Landscape indicators to discriminate successful biocontrol***

Considering these results, we divided fields into two groups depending on whether the parasitism rate (D) exceeded a predefined threshold (Dth<sub>1,2,3</sub>; see materials and methods) or was below that threshold. Parasitism rate of pollen beetle larvae was above Dth<sub>1</sub> in 28.5% of the fields, above Dth<sub>2</sub> in 21.4% of the fields, and above Dth<sub>3</sub> in 9.5% of the fields.

For parasitism rates of pollen beetle larvae, the ROC analysis demonstrated again that landscape indicators based on the proportion of woodland, the proportion of semi-natural habitats and the proportion of previous year's OSR fields with reduced soil tillage always had the highest AUC values when considering the decision thresholds Dth<sub>3</sub> (Figure 3). The mean AUC value at all scales and all decision thresholds was 0.77 for the indicator "proportion of woodland", 0.70 for the indicator "proportion of semi-natural habitats" and 0.70 for the indicator "proportion of previous year's OSR fields with reduced soil tillage", indicating that they are informative and that they had good ability to discriminate between negative and positive situations. Thus, when considering all spatial scales for each decision threshold, it seems that the indicator "proportion of woodland" is a more informative indicator than the two others as AUC values were generally higher for this indicator except for the proportion of previous year's OSR fields with reduced soil tillage at the 2000m scale. For the indicators "proportion of woodland" and "proportion of semi-natural habitats", the lowest AUC values were always obtained for the medium spatial scales (i.e. 750 m to 1250 m), indicating better discriminating ability for successful natural pest control when these indicators were computed at small and large scales (Figure 3). The AUC values for the indicator 'proportion of OSR fields with reduced soil tillage' were always higher at larger spatial scales, indicating better discriminating ability for successful natural pest control when this indicator was computed at large scales (Figure 3).

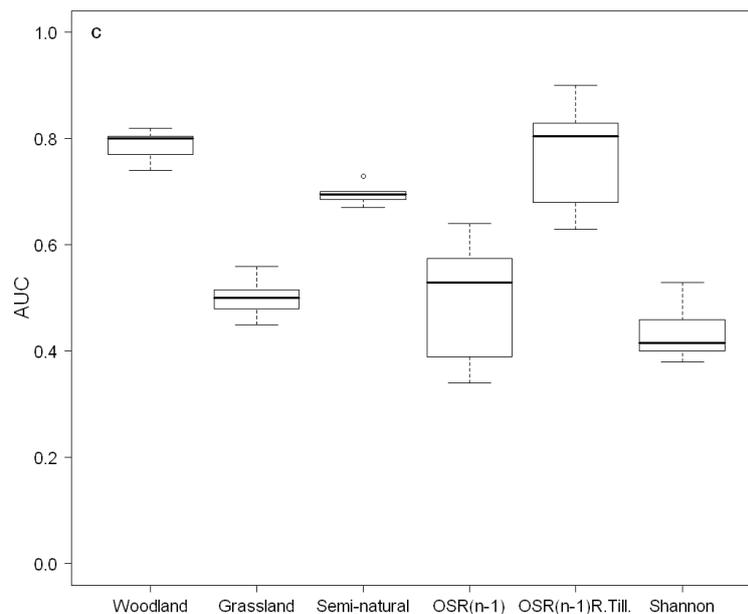


Figure 3. Values of the area under the ROC curves (AUC) for each individual indicator considering all spatial scales for high parasitism rates as function of threshold values Dthp3.

Considering all the indicators together in a logistic regression, estimated parameter values and the relative variable importance obtained for logistic regressions revealed an important positive effect of the proportion of woodland on the probability of high pollen beetle infestation to occur for each decision threshold. We also found positive effects of the proportion of woodland and the proportion of ‘previous year’s OSR fields with reduced tillage’ on the probability of high parasitism rates for each decision threshold. AUC values estimated by leave-one-out cross validation for each logistic regression are given in Table 1 for pollen beetle infestation and in Table 2 for natural pest control. Thus, AUC values obtained from logistic regression for pollen beetle infestation and parasitism rates were generally not higher than their respective values obtained for the best performing individual indicator. For parasitism rates, AUC values obtained from logistic regression at Dthp3 with landscape indicator computed at large scales were higher than the best performing individual indicator at same scales. These results were thereafter used to produce binary maps with or without successful pest control over the entire region according to the indicator value. We present here the maps obtained for the indicator proportion of woodland at only the 2000m spatial scale, as it appeared to be an indicator with good discriminatory ability (Figure 4). At regional scales, it permitted to identify the area, where specific crop management methods have to be designed to increase the biological control.

These results highlight that whatever the statistical method obtained, both local and landscape management are important elements to take into account when designing integrated pest management strategies to maximise biological control. To our knowledge, the accuracy of such landscape indicators to discriminate areas with high and low risks of pest infestations and areas able to provide successful pest control has never been examined. Mapping landscape according to landscape indicator values for predicting the risk of infestation and the potential of biological control can be a useful tool for extension services and landscape managers but it can also help scientists in formulating hypothesis about landscape functioning at large scales.

Table 1. Values of the area under the curve (AUC) of each individual indicator computed at various spatial scales and of the logistic models combining several landscape indicators computed at the same spatial scale for high pollen beetle infestation. AUC values have been calculated for three different thresholds:  $D_{th1} = 2$  pollen beetles per plant at growth stage 51;  $D_{th2} = 6$  pollen beetles per plant at growth stage 55;  $D_{th3} = 15$  pollen beetle per plant at growth stage 55. AUC was estimated by cross-validation for logistic models.

| Decision thresholds | Indicators                         | Spatial scale |      |      |       |       |       |       |       |
|---------------------|------------------------------------|---------------|------|------|-------|-------|-------|-------|-------|
|                     |                                    | 250m          | 500m | 750m | 1000m | 1250m | 1500m | 1750m | 2000m |
| $D_{th1}$           | Prop woodland                      | 0.82          | 0.83 | 0.81 | 0.79  | 0.81  | 0.82  | 0.85  | 0.85  |
|                     | Prop grassland                     | 0.51          | 0.52 | 0.51 | 0.54  | 0.54  | 0.54  | 0.53  | 0.52  |
|                     | Prop semi-natural habitats         | 0.67          | 0.76 | 0.72 | 0.74  | 0.76  | 0.77  | 0.79  | 0.78  |
|                     | Prop OSR                           | 0.38          | 0.41 | 0.43 | 0.47  | 0.48  | 0.44  | 0.44  | 0.43  |
|                     | Shannon index of habitat diversity | 0.66          | 0.54 | 0.46 | 0.45  | 0.45  | 0.44  | 0.43  | 0.44  |
|                     | Logistic regression                | 0.79          | 0.80 | 0.79 | 0.77  | 0.76  | 0.78  | 0.83  | 0.84  |
| $D_{th2}$           | Prop woodland                      | 0.71          | 0.80 | 0.81 | 0.80  | 0.80  | 0.82  | 0.82  | 0.82  |
|                     | Prop grassland                     | 0.52          | 0.58 | 0.58 | 0.56  | 0.56  | 0.57  | 0.57  | 0.58  |
|                     | Prop semi-natural habitats         | 0.61          | 0.81 | 0.82 | 0.83  | 0.84  | 0.84  | 0.85  | 0.85  |
|                     | Prop OSR                           | 0.41          | 0.41 | 0.35 | 0.34  | 0.32  | 0.29  | 0.29  | 0.30  |
|                     | Shannon index of habitat diversity | 0.56          | 0.57 | 0.52 | 0.49  | 0.50  | 0.50  | 0.50  | 0.51  |
|                     | Logistic regression                | 0.64          | 0.79 | 0.80 | 0.81  | 0.81  | 0.82  | 0.84  | 0.87  |
| $D_{th3}$           | Prop woodland                      | 0.78          | 0.83 | 0.84 | 0.85  | 0.87  | 0.87  | 0.86  | 0.85  |
|                     | Prop grassland                     | 0.56          | 0.48 | 0.48 | 0.44  | 0.40  | 0.41  | 0.42  | 0.44  |
|                     | Prop semi-natural habitats         | 0.70          | 0.78 | 0.75 | 0.75  | 0.78  | 0.80  | 0.83  | 0.84  |
|                     | Prop OSR                           | 0.45          | 0.57 | 0.53 | 0.48  | 0.48  | 0.38  | 0.38  | 0.37  |
|                     | Shannon index of habitat diversity | 0.49          | 0.56 | 0.50 | 0.45  | 0.43  | 0.43  | 0.43  | 0.43  |
|                     | Logistic regression                | 0.63          | 0.82 | 0.81 | 0.82  | 0.85  | 0.86  | 0.86  | 0.78  |

Table 2. Values of the area under the curve (AUC) of each individual indicator computed at various spatial scales and of the logistic models combining several landscape indicators computed at the same spatial scale for successful biological control. AUC values have been calculated for three different parasitism rates thresholds:  $D_{thp1} = 70\%$ ;  $D_{thp2} = 80\%$ ;  $D_{thp3} = 90\%$ . AUC was estimated by cross-validation for logistic models.

| Decision thresholds | Indicators                                    | Spatial scale |      |      |       |       |       |       |       |
|---------------------|---|---------------|------|------|-------|-------|-------|-------|-------|
|                     |   | 250m          | 500m | 750m | 1000m | 1250m | 1500m | 1750m | 2000m |
| $D_{thp1}$          | Prop woodland                                 | 0.75          | 0.74 | 0.71 | 0.70  | 0.71  | 0.72  | 0.73  | 0.73  |
|                     | Prop grassland                                | 0.53          | 0.51 | 0.48 | 0.48  | 0.48  | 0.50  | 0.50  | 0.48  |
|                     | Prop semi-natural habitats                    | 0.72          | 0.77 | 0.72 | 0.72  | 0.74  | 0.75  | 0.75  | 0.75  |
|                     | Prop previous year's OSR with reduced tillage | 0.62          | 0.61 | 0.63 | 0.66  | 0.66  | 0.67  | 0.68  | 0.69  |
|                     | Shannon index of habitat diversity            | 0.61          | 0.36 | 0.32 | 0.35  | 0.40  | 0.42  | 0.46  | 0.49  |
|                     | Logistic regression                           | 0.74          | 0.71 | 0.64 | 0.66  | 0.69  | 0.70  | 0.72  | 0.73  |
| $D_{thp2}$          | Prop woodland                                 | 0.84          | 0.83 | 0.79 | 0.78  | 0.70  | 0.81  | 0.83  | 0.83  |
|                     | Prop grassland                                | 0.38          | 0.39 | 0.37 | 0.38  | 0.39  | 0.41  | 0.41  | 0.40  |
|                     | Prop semi-natural habitats                    | 0.62          | 0.71 | 0.67 | 0.68  | 0.69  | 0.69  | 0.71  | 0.72  |
|                     | Prop previous year's OSR with reduced tillage | 0.60          | 0.60 | 0.65 | 0.70  | 0.72  | 0.75  | 0.76  | 0.77  |
|                     | Shannon index of habitat diversity            | 0.51          | 0.38 | 0.34 | 0.35  | 0.41  | 0.43  | 0.44  | 0.45  |
|                     | Logistic regression                           | 0.74          | 0.78 | 0.76 | 0.75  | 0.79  | 0.82  | 0.84  | 0.87  |
| $D_{thp3}$          | Prop woodland                                 | 0.82          | 0.78 | 0.74 | 0.76  | 0.81  | 0.80  | 0.82  | 0.80  |
|                     | Prop grassland                                | 0.49          | 0.56 | 0.52 | 0.51  | 0.51  | 0.48  | 0.48  | 0.45  |
|                     | Prop semi-natural habitats                    | 0.70          | 0.73 | 0.70 | 0.70  | 0.69  | 0.68  | 0.68  | 0.67  |
|                     | Prop previous year's OSR with reduced tillage | 0.67          | 0.63 | 0.69 | 0.79  | 0.82  | 0.82  | 0.84  | 0.90  |
|                     | Shannon index of habitat diversity            | 0.53          | 0.38 | 0.43 | 0.40  | 0.40  | 0.40  | 0.43  | 0.49  |
|                     | Logistic regression                           | 0.58          | 0.57 | 0.56 | 0.74  | 0.89  | 0.90  | 0.92  | 0.92  |

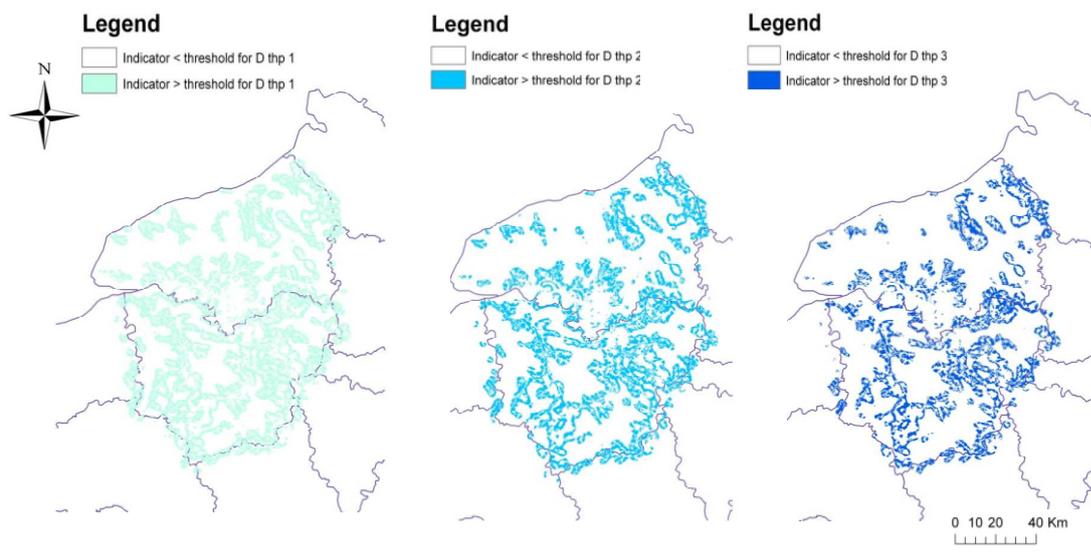


Figure 4. Binary maps of the Haute-Normandie region based on the value of the indicator proportion of woodland in the 2000m spatial scale representing arable land with parasitism rates above the three decision thresholds  $D_{thp1}$ ,  $D_{thp2}$  and  $D_{thp3}$ . White areas represent area with indicator value below the optimal threshold and coloured area represent area with indicator value above the optimal threshold.

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## The attractiveness of wild cruciferous plants on the key parasitoids of *Meligethes aeneus*

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**Abstract:** Parasitoids can play an essential role in the natural control of oilseed rape pest populations. The host-seeking mechanism of parasitoids is based on chemical cues released by the infested host plants as well as those produced by the host insects of parasitoids. In addition, plant structure and architecture can affect the host finding success of parasitoids. A small scale field experiment was conducted to investigate pollen beetle (*Meligethes aeneus* Fab.) parasitism rate by larval endoparasitoids on different wild cruciferous plants: *Brassica nigra* (L.) W. D. J. Koch (black mustard), *Raphanus sativus* L. var. *oleiformis* Pers. (oilseed radish) and *Eruca sativa* Mill. (wild rocket) compared to spring oilseed rape (*Brassica napus* ssp. *oleifera* L.). In total four species of *M. aeneus* larval parasitoids were found: *Diospilus capito*, *Tersilochus heterocerus*, *Phradis morionellus* and one unidentified. Species composition of parasitoids differed with plant species. All four species were found on *B. napus* (*P. morionellus* 47%, *D. capito* 39.8%, *T. heterocerus* 8.4%, unidentified 4.8%), three on *B. nigra* (*T. heterocerus* 68.6%, *P. morionellus* 16.3%, *D. capito* 15.1%) and *E. sativa* (*P. morionellus* 44.4%, *D. capito* 33.3%, unidentified 22.2%) and two species parasitized larvae on *R. sativus* (*D. capito* 71.4%, *P. morionellus* 28.6%).

The most common larval endoparasitoid species of *M. aeneus* in northern and central Europe are *P. interstitialis*, *P. morionellus* and *T. heterocerus*. *Brassica nigra* was the most suitable for *T. heterocerus*, the key parasitoid in most European countries. Similarly to Finland, in Estonia the most important parasitoid species in oilseed rape was *D. capito*, which was the dominant species on *R. sativum* and *B. napus*.

In conclusion, we can assume, that using *B. nigra* in seed mixtures at oilseed rape field edges may enhance species richness and abundance of parasitoids of *M. aeneus*; by preserving more species of economically important natural enemies of oilseed rape pests, it is possible to decrease chemical input and apply and uphold more sustainable pest control tactics.

**Key words:** Host plant attractiveness, host plant, *Brassica nigra*, *Phradis morionellus*, *Diospilus capito*, *Tersilochus heterocerus*

### Introduction

Oilseed rape (*Brassica napus* spp. *oleifera* L.) (Brassicaceae) is the fourth most widely grown crop in the European Union (FAO, 2010). In Estonia the area of oilseed rape grown has increased 82-fold over the past 20 years reaching 98,200 hectares in 2010 (Statistics Estonia, 2010). Products of oilseed rape are used for the food industry, in animal husbandry and most recently also for biofuel. The expansion of the area grown has provided good preconditions for population growth of the pests specialised to cruciferous plants. The most important insect pests on oilseed rape throughout Europe are the pollen beetle *Meligethes aeneus* (Fabricius) (Coleoptera: Nitidulidae), and the cabbage seed weevil, *Ceutorhynchus obstrictus* (Marsham) [syn. *C. assimilis* (Paykull)] (Coleoptera: Curculionidae) (Alford *et al.*, 2003; Veromann *et al.*, 2006a,b,c; Cook & Denholm, 2008; Veromann *et al.*, 2008; Ekbom, 2010; Williams, 2010; Veromann *et al.*, 2011). Both pests are specialised to brassicaceous plants although they have behavioural preferences for some Brassica species over others (Buechi, 1990; Ekbom &

Borg, 1996). Adult beetles of *M. aeneus* feed on pollen from plants of many different families (Fritzsche, 1957; Free & Williams, 1978; Williams, 2010), but oviposit only in buds of brassicaceous plants (Free & Williams, 1978; Nilsson, 1989; Ekbom & Borg, 1996). Females are very specific about the chemical and morphological features of the bud (Ekbom & Borg, 1996). On average, they lay 2-3 eggs per bud (Scherney, 1953), although more than 10 eggs per bud has been found by Ekbom & Borg (1996).

Management of oilseed rape pests still relies greatly on synthetic pesticides which are often applied routinely and prophylactically, without regard to pest incidence (Alford *et al.*, 2003; Williams, 2004; Thieme *et al.*, 2010). This has led to their over-use, reducing the economic competitiveness of the crop, threatening biological diversity and enhancing the pyrethroid resistance development in the pollen beetle (Hansen, 2003; Tiilikainen & Hokkanen, 2008; Thieme *et al.*, 2010). In addition, the use of pesticides does not solve the pest problem (Hokkanen, 2000) and can even increase it (Veromann *et al.* 2008); Insecticides also have detrimental effects on the parasitoids that are essential enemies of many crop pests and may act as keystone species in ecosystems (Murchie *et al.*, 1997; Thies *et al.*, 2003; Ulber *et al.*, 2010).

The foraging and subsistence of any insect is determined by their ability to find hosts. Crop location by phytophagous insects, such as the pests of oilseed rape, involves recognising the habitat of host plants and accepting a host plant; for that, the insect needs to respond behaviourally to different visual and olfactory cues. Host location mechanisms of their natural enemies, parasitoids, are grounded on stimuli from the host microhabitat (foodplant), stimuli indirectly associated with the presence of the host, and stimuli released by the host itself (Godfray, 1994). Hymenopteran parasitoids locate their hosts by the help of cues indirectly associated with the presence and activity of the host itself (Crespo & Castelo, 2008), thus they can be attracted to plants in the absence of a host. One reason for that is plants can provide food and mating site for them. Parasitoids are guided by complex blend of volatiles emitted by plants. Herbivore-induced plant volatiles (HIPVs) are produced following damage (Dicke & Baldwin, 2010). Other chemical and physical cues play their role after the insect has already found the plant (Hilker & McNeil, 2008). These other cues include plant phenology. The main plant characteristics which influence the foraging success of parasitoids are the size or surface area, structural heterogeneity and structural complexity of a plant (Andow & Prokrym, 1990).

In our experiment, four oil plant species of the family Brassicaceae were tested: *Brassica napus* L., *Brassica nigra* (L.) W. D. J. Koch, *Raphanus sativus* L. var. *oleiformis* Pers. and *Eruca sativa* Mill. *Raphanus sativus* is annual or biannual, and grows 50-100 cm high. The flowers are soft yellow, white or purple; the variety used in this study had pale violet flowers. Flower buds are yellow. Flowering starts in July and lasts about 1-1.5 months. The siliqua contain 2-3 very oil rich seeds. Seeds of wild varieties are not edible, but cultured ones are used as spice and also for snacks. The seeds of wild varieties contain up to 48% oil making it a potential source of biofuel. *Raphanus sativus* produces a large amount of biomass and is gaining importance in Europe as forage and green manure. *Brassica nigra* is an annual, common throughout Europe. It is 90-180 cm high; flowers are small and bright yellow. Pods are very numerous and about 2.5 cm long containing several small, spherical, blackish-brown, and veined seeds. It is cultivated for its flavourful seeds which are used as spice in several countries. Its oil is also used for cooking. *Eruca sativa* is an annual herb, 20-100 cm high; flowers creamy white with purple veins. The siliqua are 12-35 mm long with an apical beak, and contain several seeds. It is cultivated for its spicy young leaves as salad vegetable. In the literature of the 13th century, there is an indication of use of *E. sativa* seeds for biological control purposes – the seeds were sown together with other garden vegetables in order to

inhibit pest development (al-Qazwini, 1981; Yaniv *et al.*, 1998). It is of interest as an industrial non-food crop, due to its wide climatic and agronomical adaptation and good oil yield with high erucic acid content (Lazzeri *et al.*, 2004).

The aim of this study was to determine if and how the host plant species influence the parasitism rate of larvae of *M. aeneus* and the species composition of parasitoids.

## Material and methods

### *Study area and experimental design*

The study was carried out in an experimental field of the Estonian University of Life Sciences in 2009. The plants were grown in a randomized complete block design with three replicates of each plant species: *B. nigra*, *R. sativus* var. *oleiformis* (cv. 'Bille'), *E. sativa* (cv. 'Poker') and *B. napus* (cv. 'Mascot'). Seeds of *B. nigra* and *E. sativa* were purchased from the seed company Hansaplant LLC, seeds of *B. napus* and *R. sativus* were obtained from the seed collection of the Estonian University of Life Sciences. Each plot was 1 x 5 m with 1 m wide buffer zone of bare soil around each plot; the whole experimental field was surrounded by spring barley. Plots were sown concurrently on 7 May at 250 seeds per m<sup>2</sup>. Crop management was uniform in all plots. Plant growth stage (GS) was assessed weekly using the decimal code system of Lancashire *et al.* (1991; see also Meier, 2001) (Table 1). To compare total flower production capacity of each plant species, the number of pods (successfully developed flowers) and podless stalks (flowers which had not completed their development into pods because of lack of pollination or damage including that caused by *M. aeneus*) were counted on ten randomly-chosen plants from each plot at pod maturation stage (GS 80-83; from *B. nigra* on 11 August; *B. napus* and *R. sativus* on 18 August and *E. sativa* on 15 September, in 2009).

### *Insect sampling*

For estimation of oviposition activity and larval parasitisation levels of *M. aeneus*, all larvae were collected from the flowers of ten randomly chosen plants from each plot (at full flowering stage (GS 64-67) of each crop; from *B. napus* and *B. nigra* on July 7; from *R. sativus* and *E. sativa* on July 14). In the laboratory, the buds and flowers were dissected under a stereoscopic microscope and all larvae and/or eggs were counted; second instar larvae were dissected and any parasitoid larvae and eggs found inside were recorded and the percentage parasitism calculated. Parasitoid larvae and eggs were identified to species using the key of Osborne (1960).

### *Statistical analyses*

Data analysis was conducted with Statistica 9.1 (StatSoft Inc. USA). The means of pollen beetle larval numbers, and parasitism rates were compared using the differences of least square means; to compare the parasitism rates of *M. aeneus* larvae, the second instar larva was counted as a categorical factor; the differences between species were analysed using ANOVA and post-hoc LSD. To estimate the potential capacity to produce flowers during the whole sampling period of tested plant species, the sum of the number of pods and podless stalks per plant was calculated and to test for differences among all plant species, univariate analysis of variance (ANOVA) and post-hoc LSD-test were used.

Table 1. Sampling dates and phenological growth stages (BBCH, Lancashire *et al.*, 1991) of the four cruciferous plant species tested, Estonia 2009

| Date/Plant species | <i>Brassica napus</i> | <i>Brassica nigra</i> | <i>Raphanus sativus</i> var. <i>oleiformis</i> | <i>Eruca sativa</i>  |
|--------------------|-----------------------|-----------------------|--|----------------------|
| 26 May             | 9                     | 13 – 14               | 10   | 9                    |
| 2 June             | 14 – 21               | 21 – 23               | 13 – 21  | 12 – 13              |
| 9 June             | 21 – 22               | 23 – 30               | 13 – 22  | 21 – 23              |
| 16 June            | 31 – 39               | 39 – 51               | 23 – 31  | 23 – 27              |
| 25 June            | 51 – 55               | 55 – 62               | 35 – 55  | 27 – 51              |
| 30 June            | 61 – 65               | 62 – 65               | 51 – 59  | 27 – 53              |
| 7 July             | 65 – 67 <sup>1</sup>  | 65 – 67 <sup>1</sup>  | 59 – 62  | 27 – 55              |
| 14 July            | 66 – 68               | 67 – 69               | 64 – 67 <sup>1</sup>                           | 64 <sup>1</sup>      |
| 21 July            | 67 – 69               | 71 – 73               | 67 – 69  | 39 – 64              |
| 28 July            | 68 – 73               | 73 – 75               | 68 – 71  | 39 – 64              |
| 4 Aug              | 73 – 75               | 75 – 77               | 71 – 73  | 51 – 64              |
| 11 Aug             | 75 – 77               | 80 – 94 <sup>2</sup>  | 73 – 75  | 60 – 65              |
| 18 Aug             | 81 – 85 <sup>2</sup>  |                       | 81 – 83 <sup>2</sup>                           | 65 – 67              |
| 23 Aug             | 90 – 2                |                       | 87 – 89  | 67 – 69              |
| 15 Sept            |                       |                       |  | 80 – 83 <sup>2</sup> |

<sup>1</sup> indicates the dates when samples of *Meligethes aeneus* larvae were collected; <sup>2</sup> indicates the dates when total flower production capacity were assessed

## Results

There were no significant differences in the number of buds and flowers between *B. napus*, *B. nigra* and *R. sativus* (LSD *post-hoc*  $P = 0.17$ ;  $P = 0.22$ ;  $P = 0.21$ , respectively), but *E. sativa* had significantly fewer buds and flowers than *B. nigra* and *R. sativus* (LSD *post-hoc*  $P = 0.01$ ;  $P = 0.01$ , respectively).

Overall, the mean number of *M. aeneus* larvae per plant differed significantly with plant species (ANOVA  $F_{3,116} = 34.77$ ;  $p < 0.0001$ ; Figure 1). The mean number of larvae per plant were significantly greater on *B. nigra* than on *R. sativus* ( $p < 0.0001$ ), *E. sativa* ( $p < 0.0001$ ) and *B. napus* ( $p < 0.0001$ ) (LSD-test). Also, significantly more larvae per plant were found from flowers of *B. napus* compared with *R. sativus* ( $p < 0.0001$ ) and *E. sativa* ( $p < 0.0001$ ) (LSD-test), both of which had low numbers of larvae. *Meligethes aeneus* 2<sup>nd</sup> instar larvae per plant were found approximately 2.5-fold fewer than all larvae per plant on all tested cultures (on *B. napus* 2.5 times; *B. nigra* 2.4; *R. sativus* 2.7) except *E. sativa* where the least number of 2<sup>nd</sup> instar larvae were found (3.5 times fewer). However, their abundance was correlated with the mean number of all larvae per plant and had same statistical differences between cultures (Figure 1, ANOVA,  $F_{3,8} = 16.15$ ;  $p < 0.0001$ ).

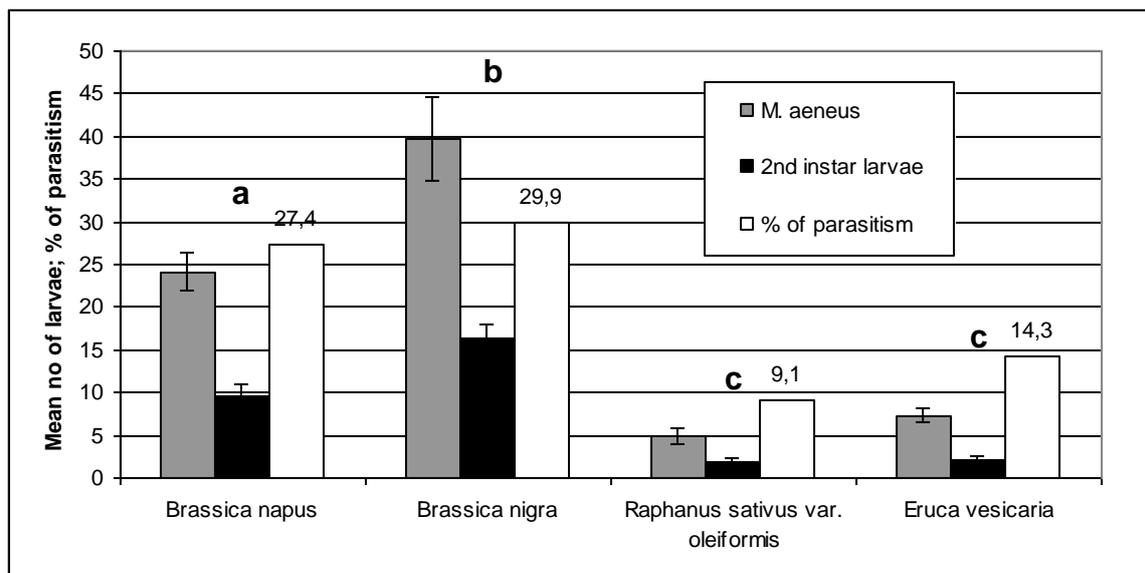


Figure 1. Mean percentage of parasitized larvae of *Meligethes aeneus* and mean number ( $\pm$  SE) per plant of *M. aeneus* larvae and 2<sup>nd</sup> instar larvae counted from different host plant flowers (BBCH 64-67). Different letters indicate significant differences between species at  $p < 0.001$  (LSD-test)

The mean number of parasitized *M. aeneus* larvae per plant differed significantly with plant species (ANOVA  $F_{3,116} = 22.66$ ,  $p < 0.0001$ ; Figure 3). They were significantly greater on *B. nigra* than *B. napus* ( $p < 0.001$ ), *R. sativus* ( $p < 0.0001$ ) and *E. sativa* ( $p < 0.0001$ ) (LSD-test). They were also significantly greater on *B. napus* than on *R. sativus* ( $p < 0.001$ ) and *E. sativa* ( $p < 0.001$ ), both of which had low numbers of parasitized larvae, and did not differ significantly. The percentage of parasitized larvae per plant was greatest in *B. nigra* (29.9%) and the lowest in *R. sativus* (9.1%) (Figure 1).

The number of *M. aeneus* larval endoparasitoids were correlated with the total number of dissected *M. aeneus* larvae ( $r = 0.879$ ) and had the same statistical differences between species as the total number of *M. aeneus* larvae and dissected larvae per plant (ANOVA  $F_{3,116} = 20.98$ ,  $p < 0.0001$ ; Figure 2).

Four parasitoid species were found from the larvae of *M. aeneus*: *Tersilochus heterocerus* Thomson, *Phradis morionellus* (Holmgren) (Hymenoptera: Ichneumonidae), *Diospilus capito* (Nees) (Hymenoptera: Braconidae) and one species that was described as “healthy-fatty-one” could not be identified in this study. In total, 271 specimens were found, most of them from *B. nigra* (172) ( $p < 0.01$ ), more than two times fewer parasitoids were found from *B. napus* (83 parasitoids); *R. sativus* and *E. sativa* both had extremely low number of parasitoids, seven and nine respectively (Figure 3). The most numerous species was *T. heterocerus* with 125 specimens, followed by *P. morionellus* with 73 specimens and *D. capito* 67 specimens; only six insects were unidentified. Species composition was very different on each plant species (Figures 3 & 4); all four parasitoid species were present on *B. napus*, the unidentified parasitoid was absent on *B. nigra*; three parasitoid species were found from larvae on *E. sativa* and only two of them on *R. sativus*. The most common parasitoid of *M. aeneus* larvae, *T. heterocerus* dominated greatly on *B. nigra* but was represented only with marginal numbers on *B. napus* and was absent on *E. sativa* and *R. sativus*. Whereas *P. morionellus* and *D. capito* were found on all plant species, with

greatest numbers on *B. napus* and *B. nigra*, both parasitoid species preferred *B. napus* over *B. nigra* (Figs. 3 & 4).

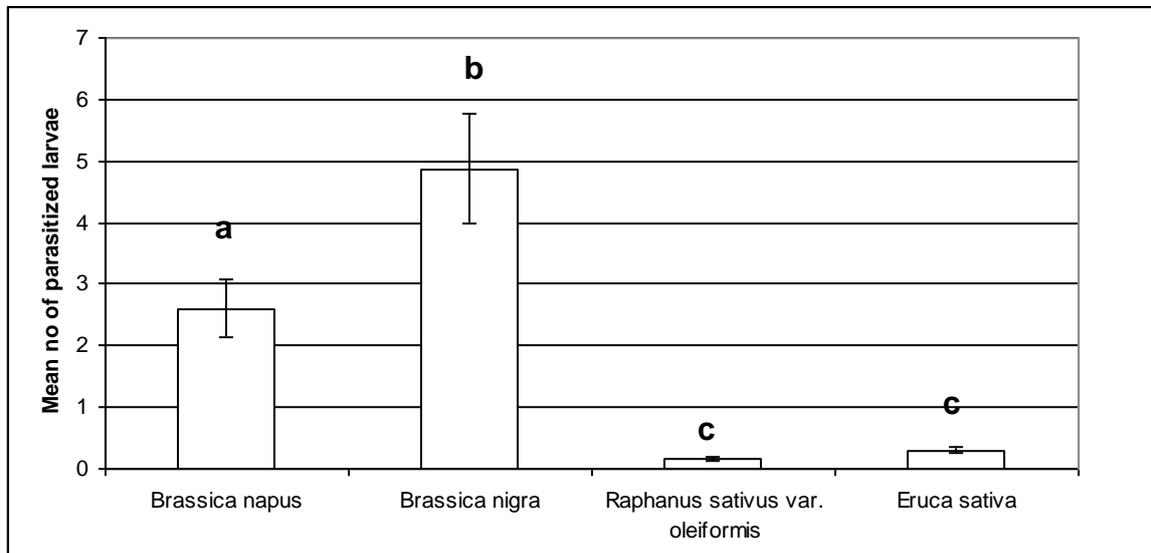


Figure 2. Mean ( $\pm$  SE) number of parasitized *Meligethes aeneus* larvae per plant. Different letters indicate significant differences at  $p < 0.05$  (LSD-test)

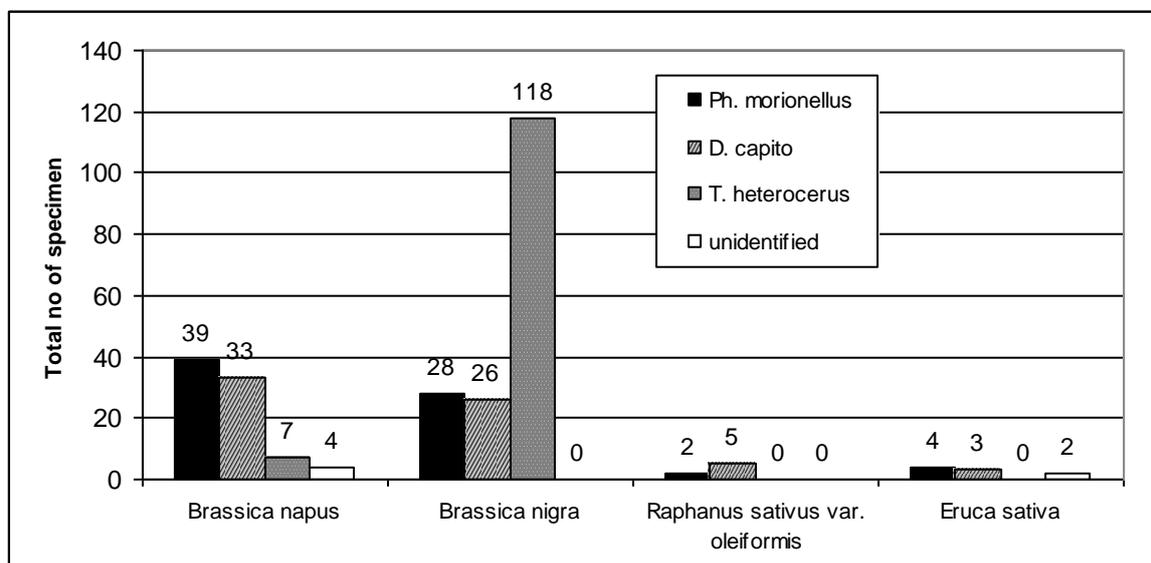


Figure 3. Species composition and total numbers of *M. aeneus* larval endoparasitoids found from 2<sup>nd</sup> instar larvae on different host plant flowers in Estonia, 2009

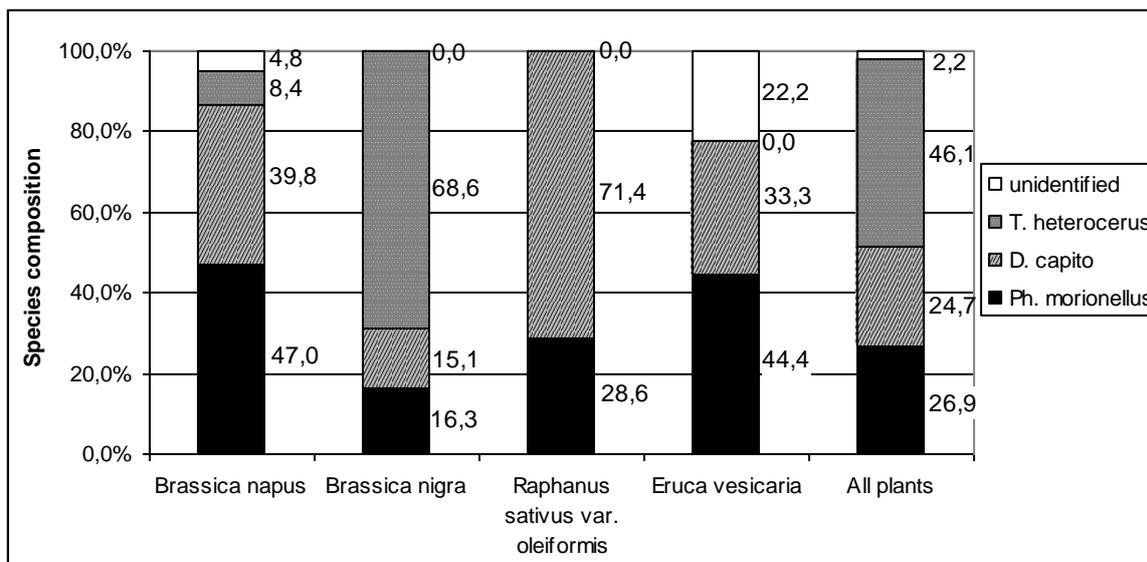


Figure 4. Species composition of the *Meligethes aeneus* larval endoparasitoid complex on different cruciferous plants in Estonia, 2009

## Discussion

In this study we found that the most preferred species tested for oviposition by *M. aeneus* was *B. nigra* upon which significantly more larvae were found than on *B. napus*, *E. sativa* and *R. sativus*. The second preferred species tested was *B. napus*, which hosted more larvae per plant than *R. sativus* and *E. sativa*. That *R. sativus* and *E. sativa* were less attractive to *M. aeneus* than *B. nigra* and *B. napus* concurs with Veromann *et al.* (2011) who showed that *B. nigra* was the most attractive plant for overwintered *M. aeneus* adults for feeding as well as oviposition. To the contrary of our field experiment results, Borg (1996), Ekbom and Borg (1996) and Ulber and Thieme (2007) reported significantly fewer larvae on *B. nigra* than on *B. napus* in laboratory conditions. Nevertheless, concerning the most unattractive plant, *E. sativa*, our findings concur with Ekbom (1998) and Ekbom & Borg (1996) who found that *M. aeneus* also preferred *B. nigra* and *B. napus* over *E. sativa*. The flight and feeding activities of adult *M. aeneus* during the inflorescence stage were similar on all tested crucifer species except *E. sativa* (Veromann *et al.*, 2011). Therefore, within the same host plant composition the adult overwintered beetles had more selective oviposition behaviour than feeding behaviour.

The abundance of 2<sup>nd</sup> instar larvae of *M. aeneus* per plant decreased almost 2.5 times on all tested crops except *E. sativa* where the decrease was 3.5-fold. It indicates that larval intraspecific competition is more severe in unfavourable host plant conditions where the food quality or quantity might not be sufficient for larval development and could be an additional stress source. Ekbom (1998) has reached the same conclusion, on poor host plant the competition is more severe and females can regulate the amount of eggs depending on the host quality. According to Hokkanen (2000) the intraspecific competition in *M. aeneus* is fiercest at the end of larval development and one turnip rape plant supplies approximately 15-20 full-grown larvae in field conditions in Finland. This concurs with our study where the mean numbers of 2<sup>nd</sup> instar larvae per plant on the attractive plants for oviposition, *B. napus* and *B. nigra*, were 10 and 16, respectively.

In total, 271 specimens from four parasitoid species were found from the larvae of *M. aeneus*: *Tersilochus heterocerus*, *Phradis morionellus*, *Diospilus capito* and one species could not be identified. The hosts were most successfully found from *B. nigra* by parasitoids. The host finding success of parasitoids was more than two times smaller on *B. napus* than on *B. nigra*. Both *R. sativus* and *E. sativa* had extremely low number of parasitoids.

The most common and widely distributed parasitoid species of *M. aeneus* in central Europe and the United Kingdom are *P. interstitialis*, *P. morionellus* and *T. heterocerus* (Winfield, 1963; Klingenberg & Ulber, 1994; Büchi, 2002; Ferguson *et al.*, 2003; Nilsson, 2003) whereas in northern Europe (Estonia, Finland, Central Sweden), where more spring oilseed rape is grown, *P. morionellus* and *D. capito* are most common (Hokkanen, 1989; Billqvist & Ekblom, 2001a,b; Nilsson, 2003; Jönsson *et al.*, 2004; Hokkanen, 2006; Veromann *et al.*, 2006b,d; Ulber *et al.*, 2010). In general, the most abundant parasitoid species in our study was *T. heterocerus*, because it was extremely abundant on *B. nigra*. Adults of *T. heterocerus* and *P. morionellus* commonly colonize the crop at the same time – at the beginning of flowering (Ulber & Nitzsche, 2006; Williams, 2006; Ulber *et al.*, 2010); they oviposit into small larvae within buds and large second instar larvae in open flowers (Nilsson, 2003). Although, the host seeking behaviour is similar in all three parasitoid species, the host finding success of females of *T. heterocerus* on *B. nigra* was predominantly more effective than of females of *P. morionellus* and *D. capito*. Parasitized larvae by *T. heterocerus* constituted approximately 70% of all infested larvae on *B. nigra*. Only 6.4% from all *T. heterocerus* specimens were found on *B. napus*. Insects are greatly influenced by growth stage of their host plant. The plants of *B. nigra* developed faster than *B. napus* and offered suitable buds for oviposition for pollen beetles and suitable host larvae for parasitoids earlier than *B. napus*. Thus, *T. heterocerus* adults might have appeared earlier in the field than other parasitoid species and laid their eggs on larvae in *B. nigra*. Female parasitoids are attracted by volatiles emitted by oilseed rape (Jönsson *et al.*, 2004; Williams & Cook, 2010), Jönsson and Anderson (2007) found that infested plants emit about 50% more volatiles (and also in a different blend) than plants which were not infested by the pollen beetle. Therefore it could also be possible that they were more attracted to volatiles emitted by *B. nigra* as this was the most infested plant species. In that case, there might be possible to use *B. nigra* to promote the presence of this parasitoid species.

The species composition of parasitoids on *B. napus*, where *P. morionellus* and *D. capito* were found in similar numbers, was in concurrence with studies by Jönsson *et al.* (2004) and Veromann *et al.* (2006b) where the most abundant parasitoid species in oilseed rape was *P. morionellus*. It was interesting that the proportion of *T. heterocerus* was only 8.4% from the total parasitoid community, because the florescence time of *B. napus* and *B. nigra* overlapped for approximately three weeks and offered suitable larvae for oviposition and female *T. heterocerus* (and also *D. capito* and *P. morionellus*) do not discriminate between host larvae that are already parasitized (Lehmann, 1969; Nitzsche & Ulber, 1998; Nilsson, 2003). *D. capito* and *P. morionellus* were found on all tested plants. Their number on *B. napus* was more than 20% (*D. capito* 21% and *P. morionellus* 28%), greater than on *B. nigra* and the number of *P. morionellus* was greater than the number of *D. capito* on both plant species. Therefore *D. capito* and *P. morionellus* could find their host larvae more successfully on *B. napus* than on *B. nigra* even the latter offered significantly more hosts.

Parasitism of pollen beetle larvae can be a major factor for the population dynamics of this pest. Levels of parasitism exceeding 50% have been reported from several European countries (Austria: Kromp & Kraus, 2006; Finland: Hokkanen, 2006; Germany: Nitzsche & Ulber, 1998; Sweden: Nilsson, 1989; Switzerland: Büchi, 2002; UK: Williams, 2006). Data collected during our study showed parasitism levels between 9-30%, which is much higher

than previously reported in Estonia (0-16%) (Veromann *et al.*, 2006a,b). Parasitism levels were highest on *B. nigra* (30%); 10% higher than on *B. napus* where it was 27%. Parasitism rates of larvae collected on *E. sativa* and *R. sativus* were nearly 3-fold lower than on *B. napus* and *B. nigra*.

## Conclusion

Methods concerning the reduction of pest occurrence by increasing abundance of their natural enemies can be an effective way to minimize chemical input. In order to attract more beneficial insects into crops there has to be enough food sources. Adult parasitoids cover their energetic needs from sugar sources, such as nectar from flowers, however not all flowers are suitable for this purpose. This study demonstrates that the use of seed mixtures with *Brassica nigra* at the field edges of oilseed rape fields can lead to a more sustainable pest control by creating a more diverse environment, preserving more species of natural enemies and enhancing the richness of parasitoid species (especially *T. heterocerus*), hereby producing the possibility to decrease the input of pesticides.

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## Factors affecting the larval parasitism of pollen beetle in Germany

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**Abstract:** Populations of pollen beetle (*Meligethes aeneus* F.) can be regulated through natural control by hymenopterous parasitoids. In particular the univoltine larval endoparasitoids *Tersilochus heterocerus*, *Phradis interstitialis* and *P. morionellus* (Hym.; Ichneumonidae) have been reported as key natural enemies of pollen beetle from crops of oilseed rape in various European countries. In this study, we investigated the species composition of larval parasitoids and the level of parasitism at various sites in Germany. Furthermore, we studied the influence of site-specific factors such as geographical latitude, regional proportion of the area grown with oilseed rape, field size, plant density, as well as effects of insecticide usage, i.e. insecticide application in bud or flower stage, number of applications averaged over the past five years and level of pyrethroid resistance in pollen beetle, on the total level of larval parasitism and the larval parasitism by individual parasitoid species.

In 2008 and 2009, second instar larvae of the pollen beetle were sampled from 36 and 42 fields, respectively, of winter oilseed rape across Germany. Larvae were dissected under a binocular microscope to detect parasitism. Subsamples of parasitized larvae were reared for identification of adult parasitoids. Factors affecting the parasitism and correlations between these factors were analysed by using multivariate statistical analyses ('tree model').

Total parasitism of pollen beetle larvae ranged between 1.6-55.9% and 1.0-81.3% in 2008 and 2009, respectively. In 2008, mean parasitism by *P. interstitialis* and *T. heterocerus* was on a similar level while mean parasitism by *T. heterocerus* was more frequent in 2009. The level of parasitism was significantly affected by geographic region in Germany, area grown with oilseed rape, field size and particularly by insecticide application during the bud or flower stage. These results suggest that larval parasitism of pollen beetle might be affected by the timing of insecticide application and additional site-specific factors.

**Key words:** Pollen beetle *Meligethes aeneus*, parasitoids, parasitism, *Tersilochus heterocerus*, *Phradis interstitialis*, *Phradis morionellus*, landscape factors, insecticide timing

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## **Host selection of *Tersilochus heterocerus* (Hymenoptera: Ichneumonidae), parasitoid of the pollen beetle *Meligethes aeneus* (Coleoptera: Nitidulidae)**

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**Abstract:** Larvae of the pollen beetle *Meligethes aeneus* Fabricius 1775, the major European pest of oilseed rape, are attacked by several species of hymenopterous parasitoids. One of the most abundant parasitoids in winter oilseed rape is *Tersilochus heterocerus* Thomson 1889. This species is known to be attracted by long-distance volatiles from oilseed rape in the bud and flowering growth stages (Jönsson, 2005). However, once a parasitoid lands on the inflorescence, it still needs to locate its host larva.

The aim of this study was to examine how parasitoid females react to odours from living pollen beetle larvae, and if there is any preference for a particular larval stage.

Olfactory bioassays were conducted to determine preferences of female parasitoids collected in the winter oilseed rape field at Alnarp near Malmö in Southern Sweden. In a Y-shaped glass olfactometer, the female wasps were allowed to choose between two airstreams carrying either purified air, or air from living immature stages of the pollen beetle. The pollen beetle larvae were tested in three size categories: Smallest stage (1-1.5 mm), b) intermediate stage (2-2.5 mm), and final larval stage (3.5-4 mm).

The tested *T. heterocerus* females showed a significant preference for the airstream carrying odours from the intermediate larval stage when tested against purified air. The females also significantly preferred the smallest larval stage when tested against the final larval stage, and the intermediate stage when tested against the final stage. This suggests that after landing, *T. heterocerus* females are able to react to volatile cues from the host, and prefer host larvae which have not yet reached the final larval stage.

**Key words:** *Tersilochus heterocerus*, olfactory bioassay, *Meligethes aeneus*, larvae, host growth stage preference

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## Effect of *Beauveria bassiana* (Balsamo) Vuillemin spray applications to control pollen beetles

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**Abstract:** The effect of *Beauveria bassiana* spray applications to control adult pollen beetles (*Meligethes* spp.) was investigated. The two *B. bassiana* isolates ART 2587 and ATCC 74040 were selected for the present study. The isolate ART 2587 was obtained from a mycosed pollen beetle adult, *Meligethes aeneus* (Fabricius), found in Switzerland in 2004 and applied both as unformulated spores and formulated in a 2% oil-based experimental formulation at a dosage of  $5 \times 10^{12}$  spores/ha. The isolate ATCC 74040 was obtained from Intrachem Bio Italia S.p.A. as the active ingredient of the product Naturalis-L, and applied at a dosage of 3 l/ha (0.5%). The *B. bassiana* spray applications were conducted in a standardized spray cabin at 23 °C, using turbo TwinJet nozzles at 4 bar pressure. Compared to untreated control or water only treatments, the *Beauveria* applications led to significantly increased pollen beetle mortality within few days after application. One week after spray application, the *B. bassiana* treatments achieved mortality levels similar to selected insecticide treatments. The results of this study show that spray applications may be a promising strategy for biological control of pollen beetle in oilseed rape. However, initial experiences with field applications suggest that the tested spray formulations have to be further optimised to achieve significant pest control under field conditions.

**Key words:** biological control, *Beauveria bassiana*, *Meligethes* spp., spray applications



# **Decision Support Systems**



## The decision-support system proPlant expert: A computer-based tool for integrated pest management used in Europe

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**Abstract:** proPlant expert is a computer-based consultation system on crop protection. Since many years the concept meets the requirements of farmers and consultants in both Germany and Europe. Meanwhile about one third of the users are from outside Germany. From March till June 2010 users carried out about 60,000 consultations with the proPlant expert online services. Altogether about 310,000 web pages were called. This high degree of utilisation shows that the unbiased and independent services proPlant GmbH offers also abroad together with local partners are appreciated by the users.

proPlant expert helps farmers and advisers to reduce the input of plant protection products to a minimum while giving them economic returns as good or even better than high-input routine sprays. The system offers assistance to users in making decisions on a range of major crops and problems: Fungicide and growth regulator application in cereals, insecticide, fungicide and growth regulator application in rapeseed as well as fungicide treatment in potatoes and sugar beet.

For crop protection in winter oilseed rape proPlant expert covers cabbage stem flea beetle (*Psylliodes chrysocephala*) in autumn and rape stem weevil (*Ceutorhynchus napi*), cabbage stem weevil (*Ceutorhynchus pallidactylus*), pollen beetle (*Meligethes aeneus*), cabbage seed weevil (*Ceutorhynchus assimilis*) and brassica pod midge (*Dasineura brassicae*) in spring. Regarding fungal diseases and growth regulators proPlant expert includes consultation on Phoma leaf spot (*Phoma lingam*) and growth regulator use in autumn and on growth regulator use in spring.

Meteorological data including a three-day-forecast, provided by meteorological services, build up the base for proPlant expert. The system analyses these weather data regarding immigration conditions, egg-laying periods and larval development of rapeseed pests, the infection probabilities of Phoma leaf spot since crop emergence in autumn and if the weather is suitable for growth regulator application in early spring.

The analyses are presented in online warning services including among others daily updated maps about relevant phenological dates of rapeseed pests, e.g. begin of egg deposition of stem weevils or good weather conditions for immigration of pollen beetles into the fields. This places farmers and advisers in a position to assess the current situation in one or several regions. Together with crop data (e.g. variety, growth stage, infestation levels/yellow trap catches) the system is furthermore able to develop a field-specific recommendation on the application of a specific chemical, if necessary. By this fewer treatments are generally needed against spring pests than with a threshold-based control strategy.

**Key words:** cereals; oilseed rape; potatoes; sugar beet; online warning service; fungicide; insecticide; growth regulator application

The computer-based decision support system (DSS) “proPlant expert” provides plant protection advice for the major oilseed rape pests in Europe: cabbage stem flea beetle (*Psylliodes chrysocephala*), cabbage stem weevil (*Ceutorhynchus quadridens*), rape stem weevil (*Ceutorhynchus napi*), pollen beetle (*Meligethes aeneus*), cabbage seed weevil (*Ceutorhynchus obstictus* (syn. *C. assimilis*)) and brassica pod midge (*Dasineura brassicae*). proPlant expert considers not only the amount of adult beetles monitored, for example by

yellow water traps but also weather data, because this plays a key role for migration, egg deposition and larval development. Based on weather data the program forecasts the start and end of the immigration period and daily conditions for flight, time needed for ovary maturation, the beginning, end and intensity of egg-laying and the progress of larval development. The results of the phenological models calculating these biological processes are shown together with weather data including a three-day forecast in graphs, so the user can review all weather-based pest activities (Figure 1).

The proPlant DSS integrates the computed data from the pest phenological models with input data about the field and the crop (e.g. wind exposure, date of emergence, growth stage, observed pest densities) and produces a field-specific risk assessment with advice on the need for insecticide application. If proPlant advises to treat, it gives specific information on suitable insecticides, optimum dates and rates for their application, and evaluates the efficacy of past applications. By using proPlant expert, optimal dates for risk assessment and treatments can be identified with minimal loss of time. Usually fewer treatments are needed than with a control strategy based only on thresholds.

In autumn, only the cabbage stem flea beetle pest needs to be considered. The task of proPlant expert here is to improve the basis for a treatment decision by identifying years when crops are at risk from damage by cabbage stem flea beetle. Since the larvae are the most damaging stage, the system estimates the potential larval density, based on known adult pest density measured by yellow water trap catches and known weather conditions. proPlant expert calculates the egg-laying period, the beginning of egg hatch and the occurrence of the second and third larval instars. These are the key points in development of the pest. proPlant expert decision making then depends on:

- total number of beetles/yellow water trap captured during the whole migration period starting with the first migration,
- analysis of weather conditions for egg production (duration and intensity of egg-laying period),
- analysis of larval development.

If high temperatures in September and October lead to a long egg-laying period in combination with a high share of egg hatch resulting in a higher larval density and early occurrence of later larval instars, insecticide treatment is advised in autumn if number of adult beetles/trap exceeded threshold. If larvae are still at the first or second instar stage in early autumn, the spraying of insecticides can be delayed even until early spring.

In spring complex situations have to be solved. The difficulty is the long immigration period of spring pests which can last approximately 2-3 months. To know about the phenological stages of the single pest is not enough. All pests need to be considered. Pest arrival on the crop is in the following sequence: first the stem weevils, then pollen beetle, then cabbage seed weevil and finally the pod midge as they differ in their temperature requirements for emergence and flight. The strategy of proPlant expert is to delay the first application for as long as possible, until later migrating pests have arrived. By this “delaying tactic” the number of insecticide applications can often be reduced.

The damaging stage of the stem weevils is the larvae. Since insecticides have no effect on the larvae inside the plants, treatment has to prevent egg-laying by the adults. The egg-maturing period and the intensity of egg production depend on temperature and hours of daily sunshine. Greater egg production can only be expected in combination with high temperatures after egg maturity is reached, and often starts weeks after the arrival of the adult beetles. In many years this consideration of egg production makes it possible to control stem weevils together with the main migration of pollen beetle and the beginning of immigration of the cabbage seed weevil. But this ‘delaying tactic’ does not work every year, for example if

pollen beetle has to be treated early in the season or if rape stem weevil is the dominant stem-weevil pest (higher risk compared with cabbage stem weevil since even low egg production can cause damage), or if egg production starts soon after first emergence because of high temperatures. Under these circumstances, early treatment is necessary and will be advised by proPlant expert. Sometimes, a second or even third treatment cannot be avoided (Figure 2).

Meanwhile proPlant expert for management of oilseed rape pests is well established not only in Germany, but also in Austria, Belarus, Czech Republic, France, Sweden and Switzerland. In the UK tests are currently being carried out with the system (Defra project LK09108; see Ferguson *et al.*, this volume). About one third of users are from outside Germany. Pest activity in crops of winter oilseed rape was monitored for several years for example in France and Sweden. The tests revealed that proPlant models compute reliable prognoses in different climates (see: Ep-01 PC demonstration: The proPlant expert decision support system for pest and disease management in oilseed rape). For example the pollen beetle phenological model produces mostly accurate results regarding the prediction of the immigration progress until flowering, depending on the season and on the region (Figure 3).

The models of the proPlant system are validated each year. When work on phenology began, there was little information on the effects of weather on population dynamics. The strategy for pest control in oilseed rape was based solely on control thresholds. Meanwhile monitoring series from nearly 20 years and from different climates now exists. During the last years the focus of further development of proPlant switched from modeling of biological processes to the evaluation of insecticide effects and on control strategies. This became essential after season 2006 when extremely high pollen beetle densities and low efficacy of treatments led to the detection of pollen beetle resistance against pyrethroids which in turn led to the registration of new insecticides (e.g. neonicotinoides).

New findings on insecticide efficacy, and results from our own field trials carried out in collaboration with the Chamber of Agriculture North Rhine-Westphalia, are incorporated into the proPlant expert system. In the insecticide database all registered products are evaluated regarding their direct (knock-down) and continuous effect (in degree days) on the different rapeseed pests. The continuous effect mainly depends on the maximum temperature (Figure 4). Treatments in very warm periods with 20 °C and above have only a very short effect. For pollen beetles however, these are optimal conditions for immigration. proPlant expert shows clearly that under these conditions field checks are needed just 3-4 days after application. Also the effect of pollen beetle resistance to pyrethroids is included in this evaluation. The proPlant database is updated each year. The assessment of the insecticide performance is done impartially.

To summarise:

- proPlant expert contains weather based phenological models for the 6 major rapeseed pests based on nearly 20 years of experience.
- proPlant expert is independent and neutral. It provides comprehensive consultation with field-specific treatment decisions and evaluation of treatment efficacy.
- The models have been validated in several European regions.
- proPlant is further developed continually.
- The internet services provided with partners become more and more popular.
- Approximately one third of users are currently from outside Germany.

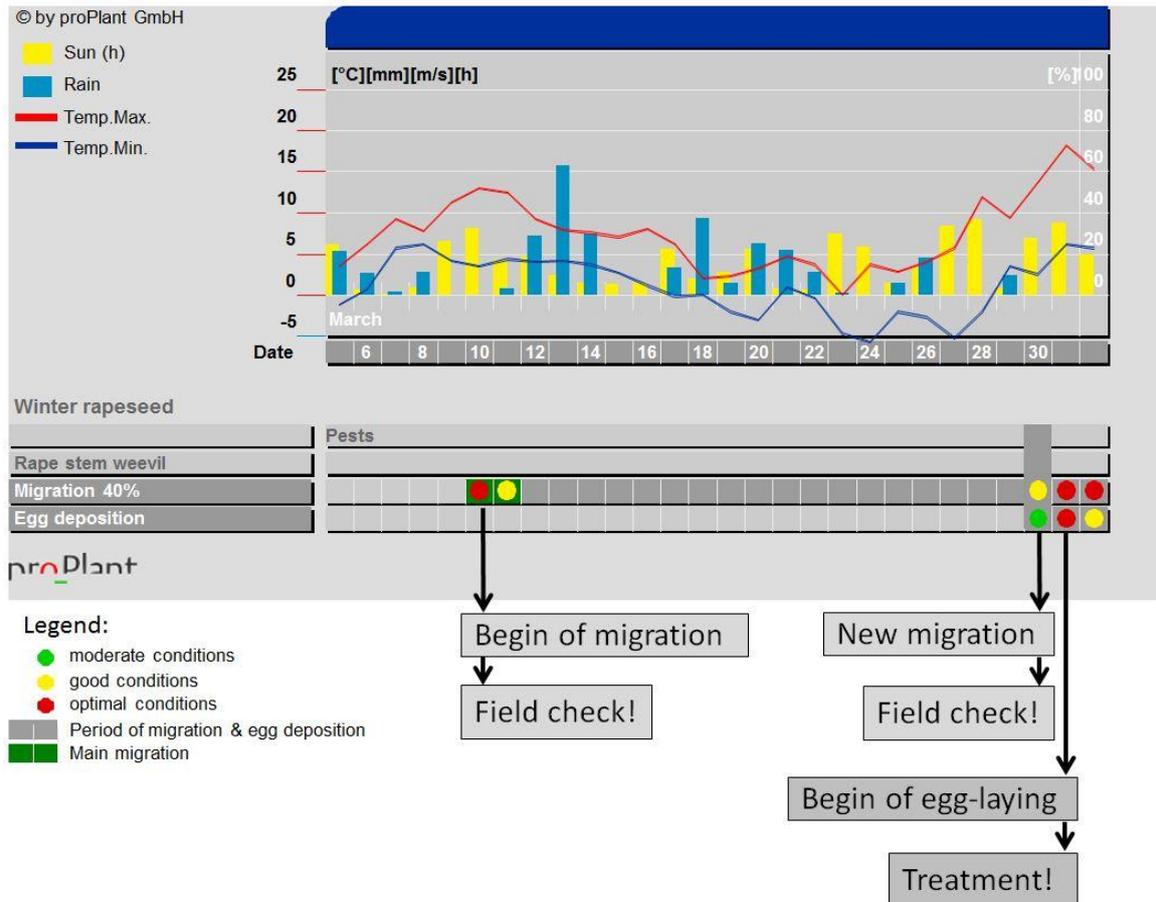


Figure 1. The phenological model results are presented together with weather data in proPlant expert pest graphs. They give on the top an overview of the weather conditions during the last three weeks and also include a three-day forecast. As shown in this example for the rape stem weevil, at first the beginning of migration in spring is identified by the system when temperatures rise. From then on for each day the weather conditions for immigration are analysed: Dark grey dots mark days with optimum conditions for migration. Light grey dots mark days with favourable and black dots with moderate weather conditions. Empty boxes indicate that migration is not to be expected. With help from the weather forecast, users of the proPlant expert. system do not miss the start of migration, i.e. facilitating placement of traps into the field in time and to check after days with good immigration conditions. After arrival in the crop, a period of time is needed for the ovaries of adult stem weevil females to mature before egg-laying starts. proPlant calculates the time for ovary maturation and the start of the egg-laying period and indicates from then on the daily intensity of egg-laying. This is essential knowledge for effective stem weevil control. Insecticides do not kill larvae in the stems. If adult weevils exceeded threshold values in yellow water traps, egg-laying needs to be prevented before they start intensive egg-laying.



Figure 2. proPlant risk assessment in spring defines four phenological key points for crop inspections and treatments:

**Key point 1** marks the start of immigration of the stem weevils and the pollen beetle. At this point, early treatment against the pollen beetle is recommended only if they exceed the control thresholds. The stem weevils do not yet require control as they are not ready to lay eggs.

**Key point 2** marks the start of egg-laying by the stem weevils. At this point, treatment may be required against the stem weevils if they exceed their threshold values in yellow water traps. The rape stem weevil starts egg-laying earlier because the process of maturation runs more quickly than for cabbage stem weevil and is more damaging than the cabbage stem weevil, as deposition of only one rape stem weevil egg per plant can cause severe stem deformation. It therefore has a lower control threshold and requires treatment earlier, before the start of egg-laying. Pollen beetle may also require control if their numbers on the crop exceed the threshold.

**Key point 3** is before the peak egg-laying period of the stem weevils. At this point, the stem weevils must be controlled at the latest if their numbers exceed threshold levels in yellow water traps. If daily maximum temperatures  $> 20^{\circ}\text{C}$  then this may coincide with the main migration of pollen beetles into the crop and they will also be killed by any treatment applied against the stem weevils. If egg-laying of stem weevils is delayed until late April, Key point 3 may coincide with Key point 4.

**Key point 4** marks the start of immigration of the cabbage seed weevil. At this point, the cabbage seed weevil/pod midge complex may require control if the seed weevil threshold is breached or if the crop is at risk of midge attack. It occurs after the main migration of the pollen beetle and during the period of egg-laying by the stem weevils, but by this time it is too late to control them.

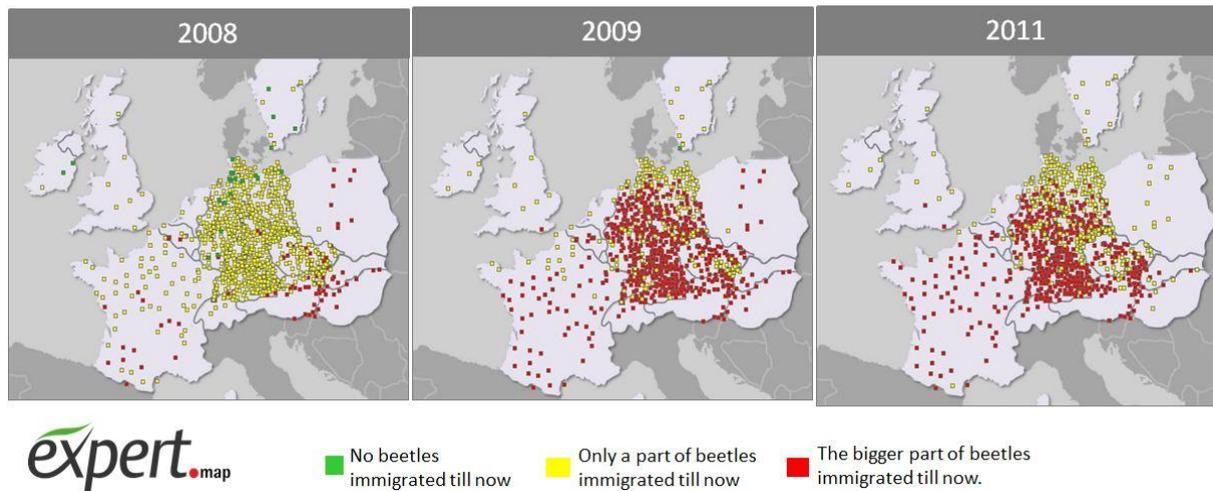


Figure 3. Pollen beetle immigration status on 20<sup>th</sup> of April according to proPlant expert: Whereas 2008 only a part of beetles could immigrate in most parts of Western Europe, in 2009 a good deal of beetles had already “arrived” in the fields in France, Germany and other regions. This was because of the extremely warm first part of April with mass immigration of pollen beetles. In England however, the weather did not follow this pattern. Like 2008, spring 2009 was more or less “typical English”. 2011 the situation on 20<sup>th</sup> of April was similar to 2009, although with some regional differences: In the South of England and the North of France the very warm spring promoted early pollen beetle immigration. The regional, nationwide or even cross-national maps in the proPlant system are updated daily. They show weather-based prognoses of relevant phenological dates, e.g. beginning of egg deposition of stem weevils or good weather conditions for migration of pollen beetles into the fields. In the season these maps provide a quick overview. Thanks to the three-day weather forecast they act as warning service.



Figure 4. The proPlant expert system also evaluates the direct and continuous effect of insecticide applications. In the proPlant pest graphs the effect is given together with weather data and phenological models. In this example the treatment against stem weevils at the beginning of the egg-laying period prevented most beetles from egg-laying. The application had a continuing effect of six days thanks to more moderate temperatures after spraying. It was also possible to control the first pollen beetles, which had good conditions for immigration on the treatment day and on the following three days. proPlant expert shows when the end of the activity of the insecticide can be expected. By this the user knows when to check the crop again, if optimal conditions for new immigration of pollen beetle are forecasted because of good weather conditions. Then the user needs to watch out and should start inspection of crops immediately.

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## **The proPlant expert decision support system for pest and disease management in oilseed rape**

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**Abstract:** The computer-based proPlant expert crop protection consultation systems offer assistance in making decisions on many problems in winter rapeseed. In autumn the system covers control of cabbage stem flea beetle and Phoma leaf spot (*Phoma lingam*) and growth regulator use. In spring it is possible to optimise insecticide applications with the aid of proPlant expert. With only few treatments a maximum efficacy can be reached against rape stem weevil and cabbage stem weevil, pollen beetle, cabbage seed weevil and pod midge.

The proPlant expert portfolio contains a range of products and services meeting the different requirements of farmers, advisors, experimentation stations, schools, colleges and universities: Commercial farmers, advisers and experimental stations use the desktop version proPlant **expert.classic**. The software offers a maximum function range: The “regional consultation” includes comprehensive graphics for planning the running crop protection season but also for final overview (e.g. of field trials) by analysis of weather data regarding infection probabilities of Phoma leaf spot, conditions for migration and egg deposition of pests and conditions for the application of growth regulators expert.classic also offers a “plot-specific consultation” to evaluate the treatment need (depending on weather analysis and field data, e.g. variety, growth stage, last treatment, infestation) including an unbiased recommendation of suitable chemicals and application rates. In addition expert.classic contains information systems on varieties (rating of lodging risk and susceptibility to Phoma leaf spot) and chemicals (e.g. evaluation of the direct and continuing effect of insecticides). **expert.com** is the personal proPlant consulting system on the internet. Two versions are available: The farmers’ version focuses on plot-specific treatment decisions, provides a selection of chemicals that are suitable for application and computes optimum dates and rates for an application to ensure optimum efficacy. The advisers’ version provides plot-specific treatment decisions and a regional consultation with personalised warning service images and export for use of images in facsimiles or e-mails.

The proPlant products **expert.basic** and **expert.map** are online warning services without field relation including proPlant prognoses derived from the analyses of weather data. Beyond Germany where multifarious plant protection services are provided together with different partners, proPlant partners also offer warning services in other European countries since several years.

**Key words:** *Brassica napus*; phoma leaf spot; pest migration; oviposition; weather data; fungicide; insecticide application; growth regulators

“proPlant expert” is a computer-based consultation system on crop protection. For many years the decision support system (DSS) has met the requirements of farmers and consultants in Germany, where the system is produced, and other counties in mainland Europe. The unbiased and independent system was developed to help to reduce to a minimum the input of plant protection products while giving users economic returns that are as good, or even better than in systems using high-input routine sprays. proPlant expert offers assistance to users in making decisions on a range of crops and problems, including insecticide, fungicide and plant growth regulator (PGR) application in oilseed rape, fungicide and PGR application in cereals as well as on fungicide application in potatoes and sugar beet.

Weather data with three-day forecasts, provided by official meteorological services in Europe, build up the base for the proPlant analyses. For fungal diseases the system finds out whether recent weather conditions were conducive or become conducive to pathogen infection. Regarding pests, proPlant expert identifies the start and end of immigration periods and daily conditions for flight, time needed for ovary maturation, the beginning, end and intensity of egg-laying and the periodicity of larval development.

Farmers and consultants receive advice adapted to the special situation in the field. To decide upon the need for chemical treatment and the best application date, the program needs inputted information about the crop (e.g. growth stage, growing conditions, previous sprays) and field assessments of disease infestation levels/rates or pest densities.

To ensure an unbiased suggestion on a specific fungicide or insecticide, the efficacy of all products and mixtures provided in the proPlant database are assessed by results that were obtained in official field trials. The system is updated on a regular basis to ensure any relevant information such as amendments to registration laws is immediately made available to users.

The development of proPlant expert started in Germany 1989 by the Agricultural Computer Science Institute in Muenster and the Westphalian Chamber of Agriculture. It has been distributed commercially since 1993. Project partners founded the proPlant GmbH in 1996. The company is scientifically and technologically supporting further development of the system ([www.proPlant.de](http://www.proPlant.de)).

In winter oilseed rape the proPlant expert consultation system provides decision support on main plant protection problems. In autumn proPlant covers advice for control of cabbage stem flea beetle (*Psylliodes chrysocephala*), phoma leaf spot (*Phoma lingam*) and growth regulation (Table 1).

proPlant expert identifies years when crops are at risk from damage by high densities of cabbage stem flea beetle larvae. In parallel the system identifies years with high risk of early phoma leaf spotting. Users of the system get field-specific decision support on both fungicide use for phoma control and growth regulation (timing, choice of fungicide) (see: Po-10 Identification of phoma risk years and regions with the decision-support system proPlant expert).

In spring the proPlant DSS offers consultation on cabbage stem weevil (*Ceutorhynchus quadridens*), rape stem weevil (*Ceutorhynchus napi*), pollen beetle (*Meligethes aeneus*), cabbage seed weevil (*Ceutorhynchus assimilis*) and brassica pod midge (*Dasineura brassicae*) (Table 1). The program takes into account not only numbers of adults (e.g. monitored by yellow traps) but also weather-based forecasts of migration periods, flight conditions and egg-laying periods. The phenological models are based on nearly 20 years of field observations on the influence of weather on population dynamics in different regions of Germany and in other European countries. By using proPlant, optimal dates for risk assessment and treatments can be identified with minimal loss of time. Usually fewer treatments are needed than with a control strategy based only on thresholds. (see: Eo-02 The decision-support system proPlant expert: A computer-based tool for integrated pest management used in Europe).

Table 1. proPlant expert crop protection solutions in oilseed rape

| <b>Autumn</b>   | <b>Benefit</b>   |
|---|--|
| <b>Phoma leaf spot and growth regulation</b>  |  |
| Weather-based risk for phoma infections following crop emergence  | Estimation of risk for early onset of leaf spotting. Advice when to begin regular inspections of crops |
| Weather-based daily phoma infection probabilities   | Identification of optimal treatment dates (infection-based)  |
| Field-specific consultation on phoma leaf spot and growth regulation  | Comprehensive decision support for fungicide/PGR use in autumn (timing, product choice, dosage)        |
| Neutral evaluation of fungicide activity against phoma leaf spot (degree days)  | Interpretation of curative and protective effect of an application (days)                              |
| <b>Cabbage stem flea beetle</b>   |  |
| Weather-based analysis of migration probabilities, conditions for egg deposition and larvae development   | Improvement of treatment decision by identification of risk years with high larvae densities           |
| <b>Spring</b>   |  |
| <b>Benefit</b>  |  |
| <b>Pests and growth regulation</b>  |  |
| Weather-based analysis of migration probabilities (rape stem weevil, cabbage stem weevil, pollen beetle, cabbage seed weevil, pod midge) and of egg deposition conditions (rape stem weevil, cabbage stem weevil) | Review of all weather-dependent pest activities (warning service, timing of field inspections)         |
| Field-specific consultation on spring pests and growth regulation   | Comprehensive decision support for insecticide and PGR use in spring (timing, product choice, dosage)  |
| Neutral evaluation of insecticide activity  | Interpretation of direct (knockdown) and continuing (days) effect of an application                    |

proPlant expert DSS is used by advisers and farmers all over Germany. During recent years it has also become popular in many other European countries (Figure 1). From March-June 2011 users carried out about 70,000 consultations with proPlant expert online services. Altogether about 300,000 web pages were called. This high degree of utilisation shows that proPlant services are appreciated by the users. Meanwhile about one third of users are from outside Germany, which reveals that proPlant models are robust and compute reliable prognoses in different climates. For example the models give a correct forecast of the much earlier immigration start of oilseed rape pests in the West and South of France compared to Germany or the UK.

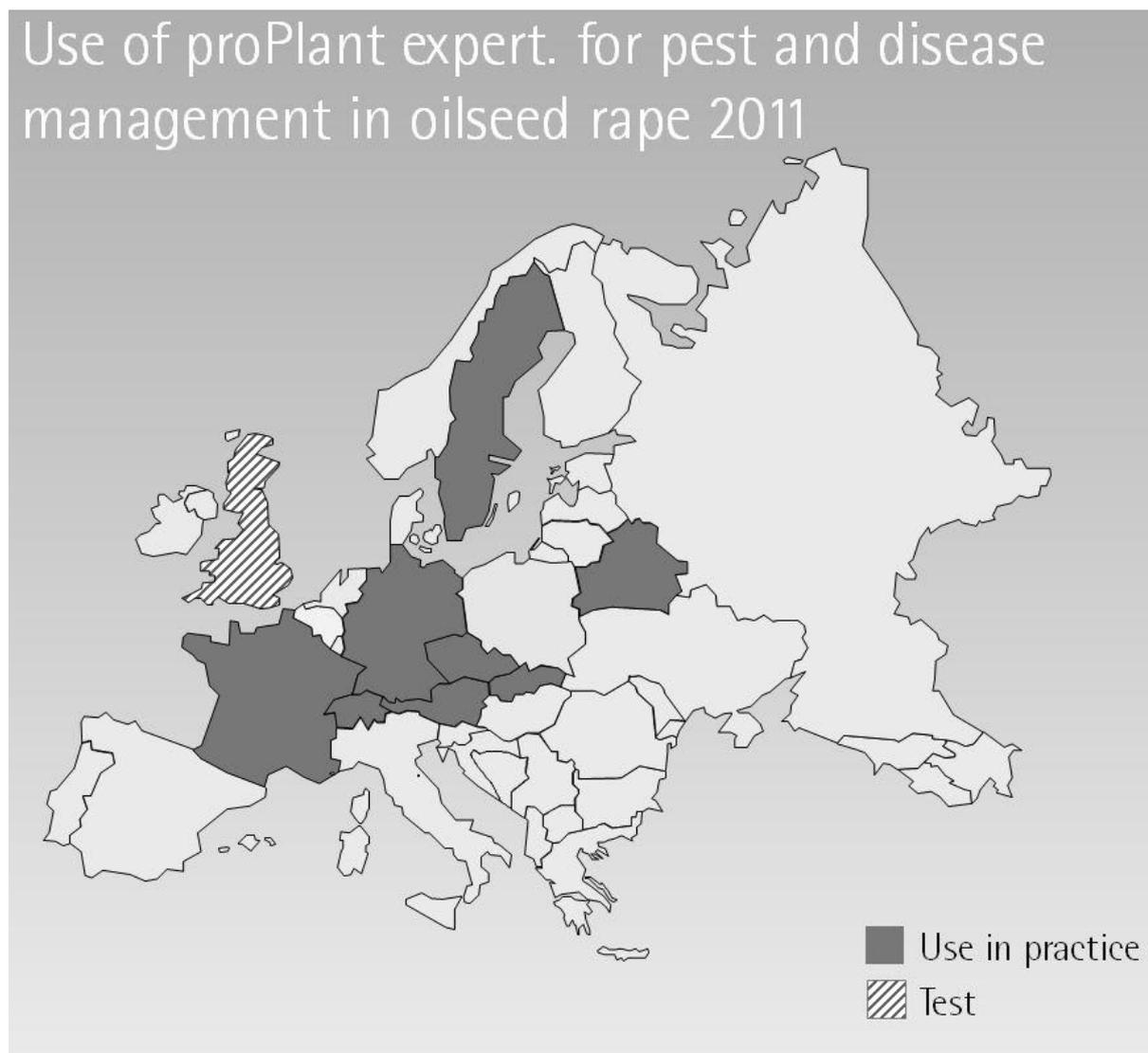


Figure 1. proPlant expert. for plant protection management in oilseed rape. The system is now established beyond Germany in: Austria, Belarus, Czech Republic, France, Slovakia, Sweden and Switzerland. In the UK, tests are being carried out with the system.

The proPlant expert. portfolio contains a range of products and services meeting the different requirements of farmers, advisers, experimentation stations and schools (Figure 2). It can be either PC-based or internet-based ([www.proPlantexpert.com](http://www.proPlantexpert.com)).

Commercial farmers, advisers and experimental stations use the desktop version **expert.classic**. The software offers a maximum function range: The “regional consultation” includes comprehensive images for planning the running of the crop protection season and also a final overview (e.g. of field trials) by analysis of weather data regarding infection probabilities of Phoma leaf spot, conditions for migration and egg deposition of pests and conditions for the application of plant growth regulators. **expert.classic** also offers “field-specific consultation” to evaluate the treatment need including an unbiased recommendation of suitable chemicals and application rates. In addition **expert.classic** contains information systems on varieties (rating of lodging risk and susceptibility to Phoma leaf spot) and chemicals (e.g. evaluation of the direct and continuing effect of insecticides).

**expert.com** is a personalised consulting system on the internet. Two versions are available: The farmers' version focuses on field-specific treatment decisions, provides a selection of chemicals suitable for application and computes optimum dates and rates for an application to ensure optimum efficacy. The advisers' version provides in addition a regional consultation with freely configurable images and export function for use of images in facsimiles or e-mails.

**expert.basic** and **expert.map** are online warning services including prognoses for fungal infection risks or migration/egg laying probabilities of pests derived from the analyses of weather data. The prognoses can be used for regional crop protection services, which do without field relation, specific treatment decision (e.g. due to variety and observed infestation) and chemical recommendation. proPlant services offered in European countries other than Germany are mainly based on these internet applications.

|  |  |  |  |  |
|--|---|--|---|---|
| <b>Features</b>                                |   |  |   |   |
| Warning service graphs (weather analysis)      | ✓   | ✓  | ✓   | ✓   |
| Warning service maps (expert.map)              | ✓   | ✓  | ✓   | ✓   |
| Field-specific consultation                    | -   | ✓  | ✓   | ✓   |
| Regional consultation                          | -   | -  | ✓   | ✓   |
| Use of graphs for e-mails and facsimilies      | -   | -  | ✓   | ✓   |
| Information system fungicides and insecticides | -   | -  | -   | ✓   |
| Information system varieties                   | -   | -  | -   | ✓   |
| Information system weather                     | -   | -  | -   | ✓   |

Figure 2. The proPlant expert product portfolio

proPlant provides expert.basic and expert.map together with local companies and advisory organisations. In Germany for example, Rapool, an association of rapeseed breeders, offers the proPlant warning services for rapeseed spring pests and phoma leaf spot in autumn on its web pages ([www.rapool.de](http://www.rapool.de)).

In Austria within a joint project by the chambers of agriculture, chemical industry, seed producers, traders and proPlant GmbH, a central platform for plant protection was developed. Through this, farmers in Austria also receive information about the current risks to the crop posed by pests of oilseed rape in their region ([www.warndienst.at](http://www.warndienst.at)).

In France, CETIOM, the technical centre for research and development of production procedures for oilseed and industrial hemp in France, provides the proPlant oilseed rape pests warning service on its homepage [www.cetiom.fr](http://www.cetiom.fr) (since 2006). In field trials it was validated that proPlant models can be transferred to all regions of France.

To set up a proPlant expert. plant protection service outside Germany, local partners usually do not need to scope with the purchase and management of local weather data. proPlant GmbH attends to provision and updating of weather data. It also performs the maintenance of the service. The application is translated into the national language. If the partners want to provide the presentation of fungicide and/or insecticide effects in the service, proPlant GmbH evaluates product efficacy. The databases are adapted to the need of the country (registered and marketed products).



## **Pest monitoring and forecasting of the cabbage stem flea beetle (*Psylliodes chrysocephala*)**

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**Abstract:** The cabbage stem flea beetle has become a local and serious pest of winter oil seed rape in the southern part of Denmark since 1992 and since 2001 the pest is widespread in all of Denmark. Attacks have peaked in cycles of seven years and this behaviour is believed to be caused mainly by cold winters and natural enemies. The beetles invade winter oilseed rape fields at crop emergence and feed on leaves but the most severe damage is caused by the larvae tunnelling the petioles and stems of plants. In Denmark, control against larval damage relies on pyrethroid insecticides and decisions on treatment are based on a threshold based monitoring of adult beetles in the field. Uncertainties are attached to a threshold based strategy of control when monitoring is of a non-damaging stage of the pest and potentially insecticides are applied without a valid need. The public aim of reducing pesticide use as well as the potential risk of pyrethroid resistance developing in the pest puts a pressure on the need to change this control strategy of “spraying and praying” towards an integrated pest management approach. The overall objective of this study is to improve the existing management strategy towards timed action when needed by compiling reliable monitoring with details on biological key points of the pest. The existing monitoring technique will be investigated in field experiments and analysed in relation to other existing or potential methods of pest and damage monitoring. Laboratory tests will be carried out to study reproduction and fecundity and to determine developmental thresholds and requirements as well as mortality factors of the egg and larval stages. The project aims to test the following hypothesis: a) egg-laying varies with temperature and temperature dependent activities and requires continuous mating, b) thermal requirement of the different stages varies to a larger extent than indicated so far, c) mortality of larvae and possibly also eggs is influenced by low temperature and very dry or wet conditions, d) first instars are most exposed to mortality factors as they migrate from the soil into plants and e) monitoring can be improved and translated into forecasting by analysing values in relation to climate and mortality of egg and larval stages.

**Key words:** Monitoring; forecasting; cabbage stem flea beetle; Integrated Pest Management

## **Are current monitoring methods for pollen beetles meaningless?**

**Matthew P. Skellern, Nigel P. Watts and Sam M. Cook**

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**Abstract:** The use of thresholds to determine when insecticide sprays are necessary is strongly encouraged, especially in the light pest populations developing insecticide resistance. Spray thresholds for pollen beetles (*Meligethes aeneus*) are given as an average number of beetles per plant. In the UK, for example, the threshold is 15 beetles per main raceme when the crop is at the damage-susceptible green-yellow bud stage, or 5/plant for a backward crop. A grower or professional crop advisor wishing to use spray thresholds can derive the average number of beetles per plant as an estimate of the population in a crop by either monitoring several plants along the edge of the crop nearest the field gate or by monitoring several plants along a transect into the middle of the field, as is recommended by the levy board (HGCA). Both methods can lead to inaccurate population estimates given that pollen beetles are unevenly distributed in the field and that they tend to infest the crop from the edge. The population estimate derived by monitoring is therefore highly dependent on where in the crop the monitoring takes place. We demonstrated this during a public open day on Rothamsted Farm and suggest that current monitoring methods for determining population estimates for thresholds are meaningless.

**Key words:** *Meligethes aeneus*; spatial distribution; spray threshold; crop monitoring

## Comparing the performance of two decision-support systems for management of pollen beetles in oilseed rape in the UK

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**Abstract:** Decision support systems (DSS) that identify the main period of risk by modeling the population dynamics of insect pests could target pest-monitoring efforts more precisely. Moreover, they are likely to increase growers' confidence in decision-making, reducing unnecessary treatments and the risk of insecticide resistance in the target pest. A phenological model-based DSS widely used in Europe, 'proPlant expert', is now being tested for pollen beetle management under UK conditions. The performance of proPlant expert is compared with that of current advice that depends on crop growth stage and temperature and is currently disseminated to UK farmers through the CropMonitor™ website and other channels. Here we report on the first two years of this study in 2008 and 2009. Weather data and the phenology of the beetles on sticky traps and oilseed rape crops across the UK were used to validate the proPlant model in UK conditions and to compare the two DSS's. proPlant expert performed well in predicting the start of pollen beetle migration, peaks of migration and the percent completion of migration. Both current advice and proPlant performed well in prompting monitoring that would detect pollen beetle threshold breaches and potential treatment decisions arising from each were identical. However, proPlant advised *c.* 20% fewer days of immigration risk and *c.* 45% fewer monitoring days than current advice. These initial findings suggest proPlant could reduce the monitoring time and effort required to manage pollen beetles according to thresholds in the UK.

**Key words:** proPlant; CropMonitor™; decision support system; DSS; *Meligethes aeneus*; phenological model; monitoring

### Introduction

There is evidence that winter oilseed rape (WOSR) crops in the UK are often treated for pollen beetles unnecessarily. Pollen beetle populations in the UK rarely exceed spray threshold levels according to data collected by CropMonitor™ (<http://www.cropmonitor.co.uk/>) yet they are the major target of spring-applied insecticides (Garthwaite *et al.*, 2011). It appears that many growers may apply 'insurance' sprays and may not follow current advice for monitoring and treating according to thresholds (AHDB 2011). A decision support system (DSS) that accurately identifies the period of risk by modelling pollen beetle population dynamics could focus monitoring. This could make use of the DSS less onerous, leading to increased take-up of the DSS and reductions in unnecessary insecticide treatments.

Advice on pollen beetle management is currently available to UK growers through the CropMonitor™ website. It provides up-to-date measurements of crop pest and disease activity in arable crops across England and acts as portal for access to a wide range of information on pests and pest risk assessment. Advice obtainable through the CropMonitor™ website is hereafter referred to as 'current advice'.

Current advice in the UK states that pollen beetles fly at temperatures of 15 °C or above. It defines the period of risk from pollen beetles to oilseed rape as green-to-yellow bud stage (BBCH growth stage 51-59; AHDB 2011). It is recommended that crops at green-to-yellow bud stage should be inspected in the headland and midfield. Spray thresholds for pollen beetle control in WOSR are set at >15 beetles per plant in a normal crop, > 5 beetles per plant in backward crops or > 2 beetles per plant in varietal associations (HGCA 2003).

'proPlant expert' <http://www.proplantexpert.com/expert/index.jsp> is a web-based DSS developed in Germany that is widely used commercially for oilseed rape in Germany, Austria, the Czech Republic, France and Sweden (Johnen *et al.*, 2010; Johnen *et al.*, this volume). This system alerts the user to the start of pest migration and its progress and it is driven by phenological models based on historical data on pest phenology related to weather. It provides local three-day forecasts of pest immigration risk that indicate whether monitoring is needed and is parameterised by data automatically downloaded from local meteorological stations. proPlant users in Germany apply less insecticide spray than those not using this system (Johnen *et al.*, 2006).

In this paper we report on the first two years of a study comparing the performance of current advice available through the CropMonitor™ website with the performance of proPlant expert in relation to pollen beetle management in the UK.

## Materials and methods

### *Field observations*

The phenology of the beetles on WOSR during the green-to-yellow bud stage was assessed at 11 fields in the east of England, one in 2008 and ten in 2009. At each field, pollen beetles were counted twice a week on 10 plants equally spaced along each of two 30 m transects into the field at the upwind and downwind edges of the crop (relative to the prevailing west-south-westerly wind). Pollen beetles were also counted twice a week on each of two yellow sticky traps mounted at 45° to the horizontal (Blight & Smart, 1999). The traps were placed 3 m into the crop and facing out of it at the upwind and downwind sides of the field.

### *Weather data*

Weather data was obtained from meteorological stations within 2 km of each sampled field. The proPlant phenological model requires daily measurements of minimum and maximum air temperature (°C), average air temperature (°C), rainfall (mm), sunshine (h) and average wind speed (m/s).

### *Assessment and comparison of DSS's*

All assessments and comparisons of DSS's were made *a posteriori*, using known pollen beetle phenology and known weather data. For both DSS's standard UK advice on spray thresholds and the susceptibility of crops to damage was followed. Monitoring days according to current advice were defined as days when temperature reached 15 °C during the green-to-yellow bud stage.

proPlant provides three-day forecasts of the start of migration, peaks in migration and the completion of migration. The degree of risk is indicated by proPlant using a traffic-light system of green, yellow and red dots indicating 'some pollen beetle migration is possible', 'good conditions for pollen beetle migration' and 'optimal conditions for pollen beetle migration', respectively. These dots are displayed beneath a graphical display of weather parameters (Figure 1) and an estimate of the percent completion of migration is also given.

proPlant advises that monitoring is necessary only in periods when yellow or red dots are forecasted. If a contiguous series of days with red or yellow dots occurs, monitoring is needed only every third day and the last day in the series.

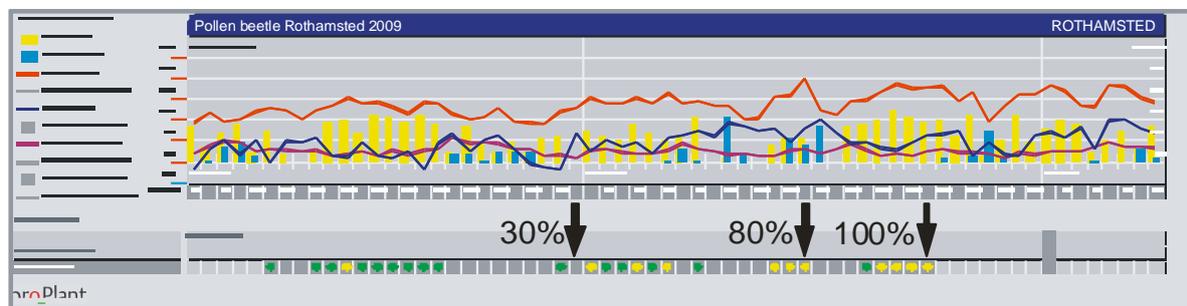


Figure 1. Example of proPlant model output, with % completion of pollen beetle migration superimposed, for Great Knott field at Rothamsted in 2009.

Advice derived from the two DSS's was related to the phenology of pollen beetles in the field and to any breaches of the two-beetle, five-beetle and fifteen-beetle per plant thresholds. The performance of each DSS was assessed and compared according to five criteria:

- the reliability with which the start of migration was indicated
- the reliability in prompting monitoring that would detect threshold breaches
- management decisions (insecticide applications) potentially resulting
- the number of days of immigration risk indicated
- the number of monitoring days advised

## Results

### *The dataset*

Pollen beetle immigration to fields in 2008 and 2009 was sufficient to provide a good test of DSS performance. Although pollen beetle numbers did not exceed 15 per plant at any of the 11 fields selected for this study, the two and five beetles per plant thresholds were breached at eight and five fields, respectively. At only three sites were no thresholds breached.

An example of the test dataset from one field is shown in Figure 2. Here the immigration risk advice from proPlant is indicated by grey and black spots, representing green and yellow indicators, respectively. No red indicators (optimum conditions for immigration) were displayed by proPlant for any field. Pollen beetle data from the site is displayed as a bar chart beneath a line representing daily maximum temperature.

## Assessment and comparison of DSS's

### *Detecting the start of pollen beetle migration*

proPlant indicated the start of the period of pollen beetle migration risk consistently well. Pollen beetles were often caught earlier on yellow sticky traps than they were detected on plants (mean  $3.8 \pm 1.10$  days earlier,  $n = 11$ ) and trap catch was therefore taken to be the more sensitive indicator of the start of migration. In nine out of the 11 test fields, monitoring began before the first migration. In all nine of these fields, the first pollen beetle catches were accompanied or preceded by a proPlant green dot, whereas temperature had by then reached  $15^\circ\text{C}$  at only five of them. This suggests that the proPlant model predicts pollen beetle flight more accurately than does the simple temperature-based rule provided by current advice.

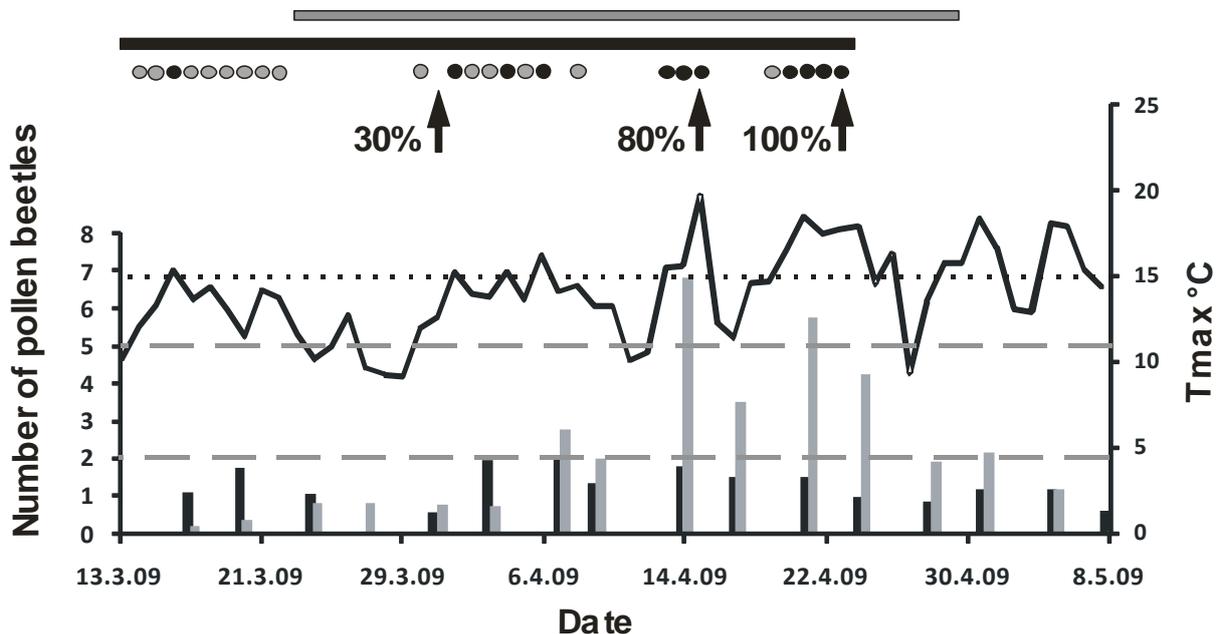


Figure 2. Numbers of pollen beetles counted at Great Knott field at Rothamsted in relation to daily maximum air temperature and indicators of pollen beetle immigration risk from proPlant. Vertical black bars = log no. pollen beetles per sticky trap; vertical grey bars = number of pollen beetles per plant; horizontal grey dashed lines indicate the 2 beetle per plant and 5 beetle per plant spray-thresholds; black plotted line = daily maximum air temperature; horizontal dotted line =  $15^\circ\text{C}$ ; percentages indicate the proportion of the pollen beetle population estimated by proPlant to have migrated by the times indicated by black arrows; large grey dot = proPlant indicates that some pollen beetle migration is possible; large black dot = proPlant indicates good conditions for pollen beetle migration; horizontal black bar = period proPlant indicates that immigration is possible given the right conditions; horizontal grey bar indicates the duration of bud stage when plants are at risk.

### *Detecting breaches of thresholds*

Current advice and proPlant were equally good at detecting immigration peaks, both maximum temperatures of  $15^\circ\text{C}$  or above and yellow proPlant spots corresponding well with peaks in numbers of pollen beetles on plants and on sticky traps (e.g. Figure 2). Recognition of a breach of the five beetle per plant threshold would have been delayed on average by a

day if using proPlant, due to its advice that daily monitoring is not necessary (Figure 3a). A small average delay in recognising breaches of the two beetles per plant threshold was also observed, both for current advice and for proPlant. This was due to one field where a threshold breach was observed but the local weather station did not record conditions suitable for migration, presumably because of very local differences in microclimate. Inaccuracies in meteorological data are likely to affect the accuracy of the prognoses from both DSS's, underlining the importance of the using of local measurements.

### ***Management decisions***

The number of insecticide treatments potentially triggered in the 11 fields would have been identical whether monitoring according to current advice or according to proPlant (Figure 3b).

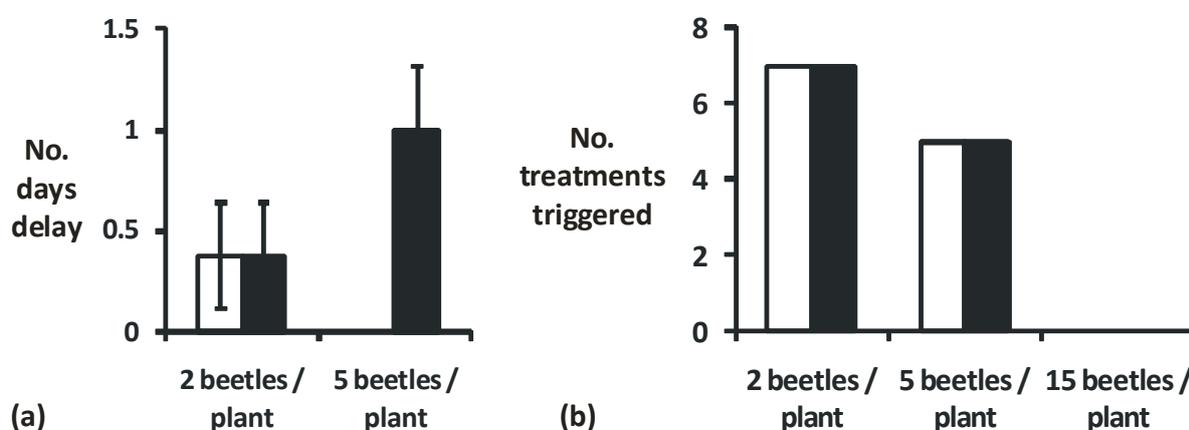


Figure 3. a) Mean  $\pm$  SEM number of days delay before breached thresholds are recognised as a result of monitoring prompted by current advice and by proPlant. (b) Numbers of insecticide treatments potentially triggered at different thresholds as a result of monitoring prompted by current advice and by proPlant.

Key:  current advice;  proPlant

### ***The number of days of immigration risk and monitoring advised***

ProPlant consistently indicated about 20% fewer days of immigration risk prior to the breaching of control thresholds than did current advice based upon a 15 °C flight threshold (Figure 4). This reduction was in part because proPlant indicated that pollen beetle migration was complete at two fields before the end of the green-to-yellow bud stage, thus allowing monitoring to stop. At both of these fields, numbers of pollen beetles counted on plants declined after proPlant advised that migration was complete (e.g. Figure 2) suggesting that the DSS accurately modelled the percent completion of migration. proPlant also identified fewer suitable days for pollen beetle migration during the period before migration was complete (e.g. Figure 2). Numbers of monitoring days advised by proPlant were even further reduced than migration risk days, being 45-50% fewer than by current advice (Figure 5). This is due to proPlant's advice that monitoring need only be done on every third day of a contiguous series of days suitable for migration.

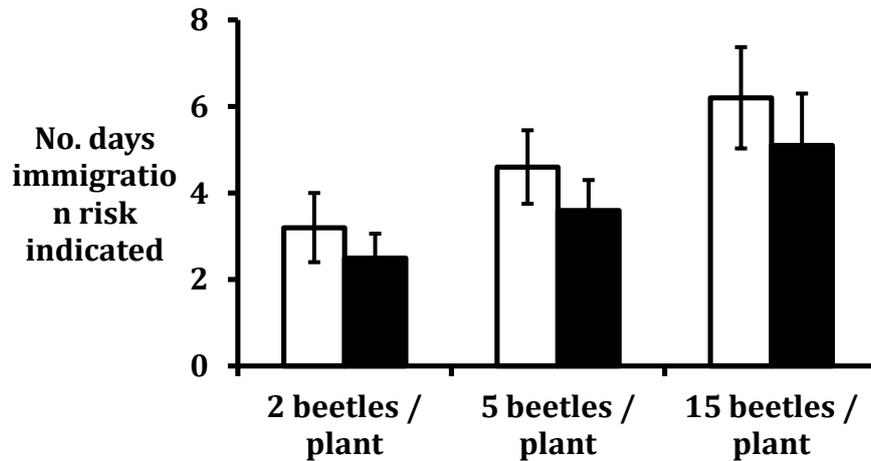


Figure 4. Mean  $\pm$  SEM number of days of pollen beetle immigration risk indicated by  $\square$  current advice and  $\blacksquare$  proPlant before each threshold is breached or to the end of bud stage (whichever period is shorter).

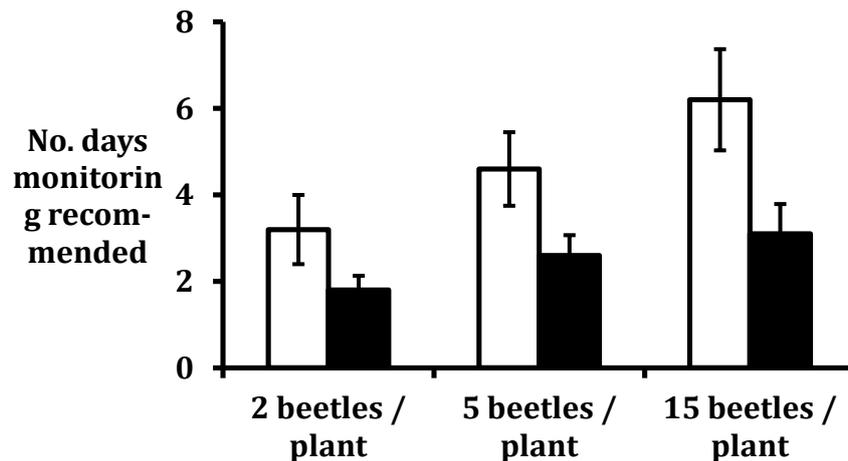


Figure 5. Mean  $\pm$  SEM number of days of pollen beetle monitoring recommended by  $\square$  current advice and  $\blacksquare$  proPlant before each threshold is breached or to the end of bud stage (whichever period is shorter).

## Discussion

The number of days of pollen beetle migration risk advised by proPlant was reduced by *c.* 20% compared to current UK advice, yet immigration events were still accurately identified. proPlant's phenological model appears to be accurate under UK conditions and to provide a more sophisticated forecast of migration risk than does maximum temperature alone, allowing monitoring to be targeted. A marked reduction in monitoring effort of 45-50% compared to current advice is achieved when also taking into account proPlant's advice to monitor only every third day during contiguous days suitable for migration.

Both DSS's performed reassuringly well in prompting monitoring that would detect breaches of spray thresholds for pollen beetles in WOSR. Moreover, management decisions resulting from each were the same. The use of proPlant would on average have engendered a day's delay in the detection of breaches of the five pollen beetle per plant threshold, due to less frequent monitoring during contiguous days of migration. It seems likely that this small delay would be accompanied by little additional risk to yield, given the compensatory ability of the crop, and would be outweighed by the benefit of using the DSS.

All assessments and comparisons of DSS's presented here were made *a posteriori*, using known weather data and known pollen beetle phenology. However, the reliability of a 3-day migration risk forecast must depend to a great extent upon the reliability of weather forecasting, both for current advice and for proPlant. Further work will compare the performance of the two DSS models in real-time using three-day weather forecasts. Data from c. 30 additional sites from 2010 & 2011 will also be analysed.

These initial findings suggest that proPlant expert reliably models pollen beetle phenology in the UK and that its introduction to the UK could reduce the monitoring time, effort and cost required to assess pollen beetle infestations according to thresholds. This could in turn increase DSS uptake by farmers, leading to better targeting of insecticides, reductions in insecticide use and costs and smaller risk of insecticide resistance.

## Acknowledgements

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## Effects of monitoring position and time of day on pollen beetle numbers in crops of oilseed rape

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**Abstract:** This study investigates the relationship between pollen beetle (*Meligethes aeneus* Fab.) immigration into an oilseed rape field, the position of the monitoring sample and weather conditions. The knowledge generated will compliment other studies that aim to refine monitoring systems for pollen beetle risk assessment. Better monitoring methods will provide farmers with a more targeted approach to applying insecticides. This may result in fewer applications which would have both economic and environmental benefits. The study was carried out in a winter oilseed rape field on Rothamsted Farm (UK) over five days in mid April 2011, towards the end of the immigration phase of pollen beetles into crops. Beetle population monitoring (a 30 m transect walk over which 10 plants were assessed) was completed every hour on each of the four sides of the field.

The transect position (side of the field) had a significant effect on the mean number of pollen beetles per plant which varied over time. Although temperature varied over the study period, it did not affect the number of pollen beetles per plant. When the data from transects were clustered into five groups according to the distance from the crop edge, the abundance of pollen beetle depended on the distance from the crop edge. These preliminary data will help to improve recommendations for best monitoring practice but additional replicated studies are needed before firm conclusions may be drawn from this work.

**Key words:** *Brassica napus*, *Meligethes aeneus*, monitoring, pest distribution, threshold detection

### Introduction

Oilseed rape is increasing as an economically important crop to growers in Europe. Rising worldwide demand has led to increased production; from 18 million tonnes in 2008 to over 21 million tons in 2009 (Eurostat, 2010).

The crop is attacked by a variety of insect pests; the most abundant throughout Europe is the pollen beetle (*Meligethes aeneus* Fab.) (Alford *et al.*, 2003; Williams, 2010). The majority of damage is caused by adult feeding on pollen within the buds which causes abscission of buds and results in podless stalks and a consequent reduction in yield (Gould, 1975; Free & Williams, 1978; Nilsson, 1988). Such damage has been reported to lead to yield losses of up to 70% on untreated spring oilseed rape crops (Nilsson, 1987).

The management of the pollen beetle relies greatly on synthetic insecticides (Walters *et al.*, 2003) as these are affordable and easily available. Insecticide treatments are often applied prophylactically without monitoring the actual pest occurrence in the crop (Alford *et al.*, 2003; Williams, 2004; Thieme *et al.*, 2010). According to Menzler-Hokkanen *et al.* (2006), over 80% of oilseed rape crops in Europe are always treated with insecticides and in the UK only 30% of oilseed rape growers sprayed selectively depending on beetles' occurrence during 2002-2003. Use of prophylactic sprays in this way increases selection for insecticide

resistance, limits the efficiency of the spray and negatively affects non-target organisms; in some cases resulting in an increase of the pests' population size (Veromann *et al.*, 2008).

The development and spread of pyrethroid resistance in populations of pollen beetles (Slater *et al.*, 2011; Heimbach & Müller, 2012; Nauen *et al.*, 2012; Šmatas *et al.*, 2012) has increased the urgency of the need to develop reliable integrated pest management methods to reduce prophylactic insecticide applications and to raise farmers' awareness of threshold levels and their confidence to use them.

The economic or action threshold is the pest population density (or level of injury) at which control action should be initiated to prevent an increasing pest population (or injury) from reaching the economic injury level (Stern *et al.*, 1959). The current threshold levels for pollen beetle differ remarkably for different countries in Europe ranging from 1-15 beetles per terminal flower/raceme/main raceme/plant (Richardson, 2008). To determine whether or not treatment is necessary according to action thresholds, monitoring of pest abundance in the crop should be done.

Several different methods have previously been used to monitor pollen beetle abundance on crops: direct counting on plants (Nilsson & Andreasson, 1987), sweep netting (Free & Williams, 1979), beating racemes into tray (Cooper & Lane, 1991), suction sampling (Alford, 1979), water traps (Free & Williams, 1978) and sticky traps (Smart *et al.*, 1996). All monitoring methods have their limitations but as the beating method does not require specialist equipment, it is the easiest and the most frequently recommended sampling method. Regarding where in the crop to carry out the monitoring, the recommendation in the UK is to sample at least ten plants along a 30 m (minimum) transect from the middle of the headland towards the centre of the crop (HGCA, 2012). Other European countries have differing methods for example in Estonia, where fields are usually small, monitoring along a 'Z' pattern across the field is recommended so that the sides of the field and the centre are monitored (Kaasik, personal observation). However, these methods have serious limitations. They are very time consuming to do properly and population means are affected by the uneven distribution of beetles in the field. Beetles fly upwind into crops (Williams *et al.*, 2007) and studies of pollen beetle distribution in the field have shown that pollen beetle adults are more abundant at the edges of the crop compared to the centres (Free & Williams, 1979; Veromann *et al.*, 2006a) and Ferguson *et al.* (2013) showed differences among field edges.

The time of monitoring assessments may also be important as beetle abundance may vary during the day leading to over- or underestimation. Studies have shown that immigration into a field occurs during daylight hours with the peak at mid-day (Ferguson *et al.*, 2003).

The aim of this study was to investigate the effect on monitoring outcome of plant position in the field (in particular the effect of monitoring plants at the edge or towards the centre of the crop), as well as the time of day and temperature during the sample.

## **Material and methods**

### ***Study area***

The numbers of pollen beetles per plant were monitored on a crop of winter oilseed rape (cv. Astrid) on Rothamsted Farm, Hertfordshire, United Kingdom. The crop area was 100 x 100 m (1 ha). The field was boarded with hedgerows on the north and west sides and a small clump of trees was situated near the south-eastern side of the field. The field sloped slightly downhill towards the northern end of the field. The neighbouring crops were spring beans to the east, and winter oilseed rape was to the west beyond the tree line. A patch of bare cultivated soil was to the south and recreational parkland was to the north. The growth stage (GS) of the crop

at the start of the study was 55-57 at the south and east sides of the crop, 57-59 at the west side, and 59 at the northern end (BBCH-code, Lancashire *et al.*, 1991).

### ***Transects***

A transect walk to estimate the number of pollen beetles per plant in the crop was conducted hourly from 07:00 to 19:00 (in total 13 counts per day) on five days between April 14th-20th (excluding April 16th and 17th) 2011. Transect walks were performed on each of the four sides of the field (orientated north, south, east and west). On each transect, one plant was sampled every three metres while walking in a straight line 30 metres into the crop towards the centre from the crop edge (Figure 1). All beetles from the main raceme of each plant were counted using the ‘beating into a tray method’ (Cooper & Lane, 1991). After assessments in the transect were completed, the assessor returned to the field edge by following exactly the same route to minimize crop disturbance and any related effect on the next transect count. The first transect count on each side of the field was done in the middle of the field edge, and each one after that on alternate sides 1 m apart from the previous one (Figure 1). Crop GS was recorded for each side of the crop daily.

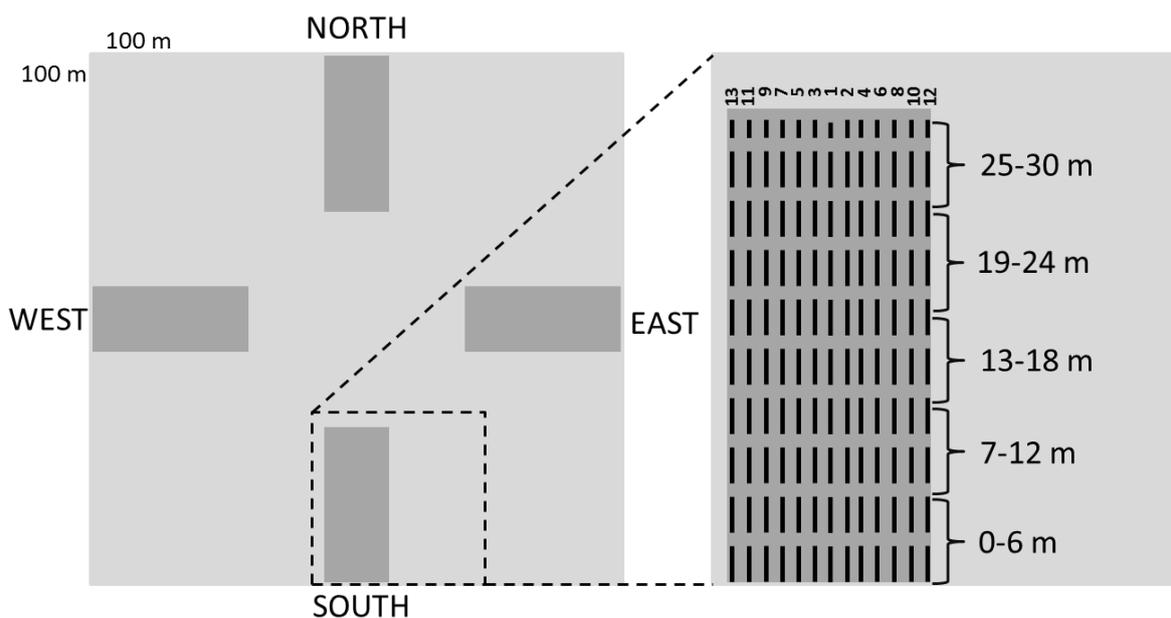


Figure 1. The positioning of transects in the test field. The four rectangles indicate the approximate transect positions; numbers 1-13 indicate the order of transect counts; 6m ranges indicate five ‘segment’ positions along the 30 m sampling transect.

### ***Meteorological data***

Air temperature (°C), wind speed and direction were recorded hourly over the five-day study period using Rothamsted’s automated weather station.

### ***Statistical analysis***

The data were analysed using a mixed model (REML) analysis (GenStat 14<sup>th</sup> Edition; VSN International, 2011) where the data were transformed using  $\log(x+1)$ . An analysis was

performed that accounted for the difference sources of variation: variation associated with the side of the field, distance from the edge of the field, day and time of day. To assess the effect of sample position along a transect, a term was included in the model where the counts were combined into five, 6m 'segments' based on the distance from the crop edge as follows: 0-6 m, 7-12 m, 13-18 m, 19-24 m and 25-30 m (Figure 1). Model terms were also included to assess differences between transect positions and days. The effect of the meteorological variables such as temperature, wind speed and wind direction were examined by using covariates within the model.

## Results and discussion

Wind direction and speed changed considerably over the study period. The wind direction moved from a predominantly south-westerly direction on day 1 towards a north-easterly direction on day 5. However, these factors were found not be important in the analysis, possibly because they were confounded by day. The air temperature was lowest at 07:00 and generally increased to 14:00 hours and cooled thereafter (Figure 2). The temperature between days varied up to 10 °C. The temperature during morning and noon transect counts during the same day also varied; differences were up to 13 °C. However, the morning temperatures were more similar than temperatures observed at 14:00.

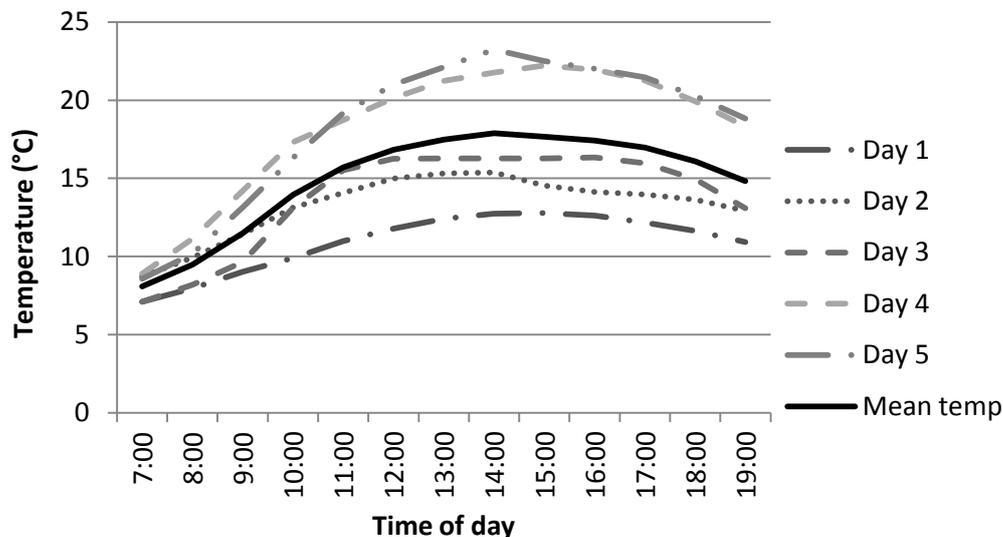


Figure 2. Air temperature (°C) recorded at the times of the transect measurements over the five day study period.

Despite the variable temperatures, temperature did not affect the mean number of pollen beetles per plant along the transect segments ( $F_{1,59} = 0.26$ ;  $p = 0.610$ ). The transect position (side of the field) had a significant impact on the mean number of pollen beetles per plant over the whole study period ( $F_{3,37} = 8.04$ ;  $P < 0.001$ ) and there was an interaction of this with day ( $F_{12,101} = 7.23$ ;  $P < 0.001$ ) (Figure 3). This seemed to be caused by a smaller number of beetles on plants in the northern part of the crop on days 4 and 5 (Figure 3). These plants had

the most advanced growth stage and days 4-5 corresponded to when these plants started to bloom (GS 60-61) while the other sides of the field were at the yellow bud stage (GS 57-59). As the trial was not replicated and no other parts of the crop were at the same growth stage, it is difficult to attribute these differences to crop growth stage or other factors related to that particular side of the crop. However, pollen beetles respond to the scent of different parts of oilseed rape flowers (Cook *et al.*, 2002) and distinctly prefer odour of flowering plants over the scent of buds (Cook *et al.*, 2007). Therefore, the current study results could suggest a stronger influence of surroundings including landscape elements (this side of the field was bordered by a hedgerow) compared to plant developmental stage.

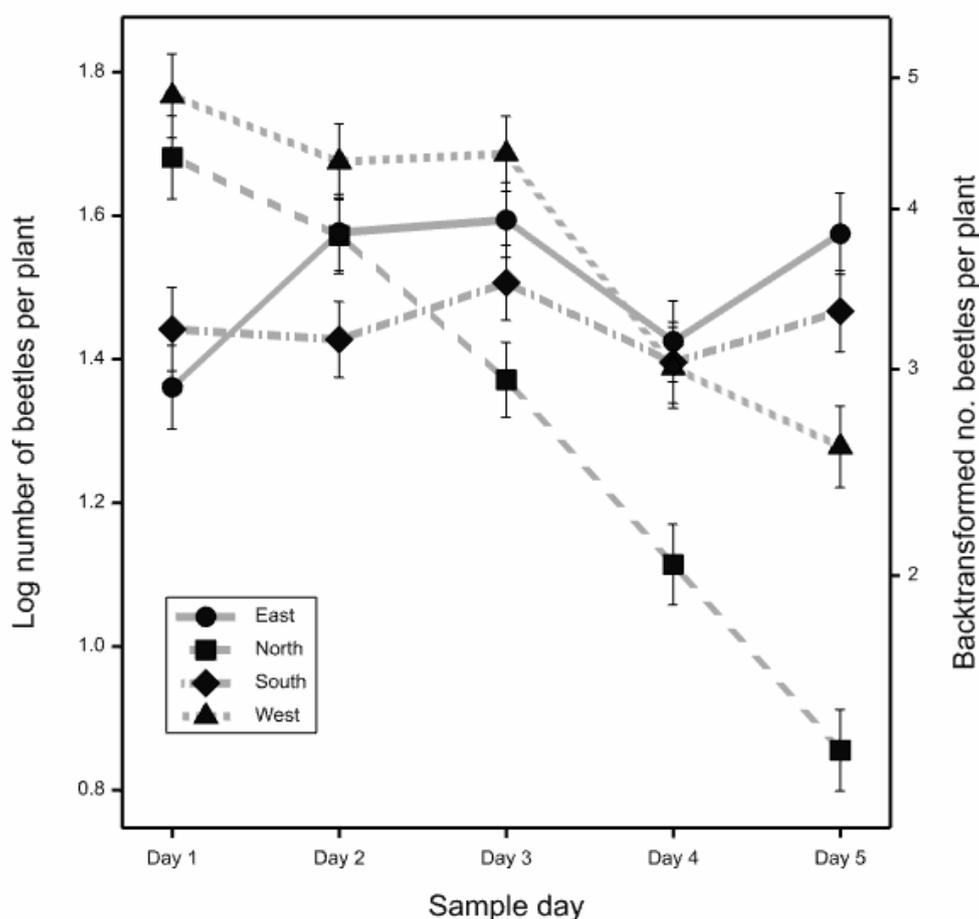


Figure 3. The log (x+1) transformed predicted mean ( $\pm$  e.s.e) number of pollen beetles per plant for four transect positions in an oilseed rape crop on five days towards the end of the immigration phase.

The mean number of pollen beetles per plant was significantly influenced by the distance from the crop edge ('segment') ( $F_{4,20} = 3.76$ ;  $p = 0.019$ ) and an interaction between segment and day was also detected ( $F_{16,80} = 2.15$ ;  $P = 0.013$ ); on days 1 and 2 there was an increase in beetle numbers on plants from the crop edge towards centre but this was not observed on days 3 to 5 when the numbers were more constant (Figure 4). These results are contrary to Free & Williams (1979) who found that the pollen beetles' abundance decreased rapidly from 20

meters from the crop edge. The differences may be caused by the differences in timing of the study period, as the immigration period in our work was towards the end of immigration the majority of pollen beetles could have been already spread over all of the crop area. However, as the experiment was not replicated the influence of temperature (it was cooler on days 1 and 2 than 3-5) and wind (direction and strength on day 3 in particular was different to the other days), as well as crop growth stage may have influenced this result. However, our results support those of Ferguson *et al.* (2003) who found that the pollen beetle spatial distribution in the field is complex with differing, irregular patterns of aggregation.

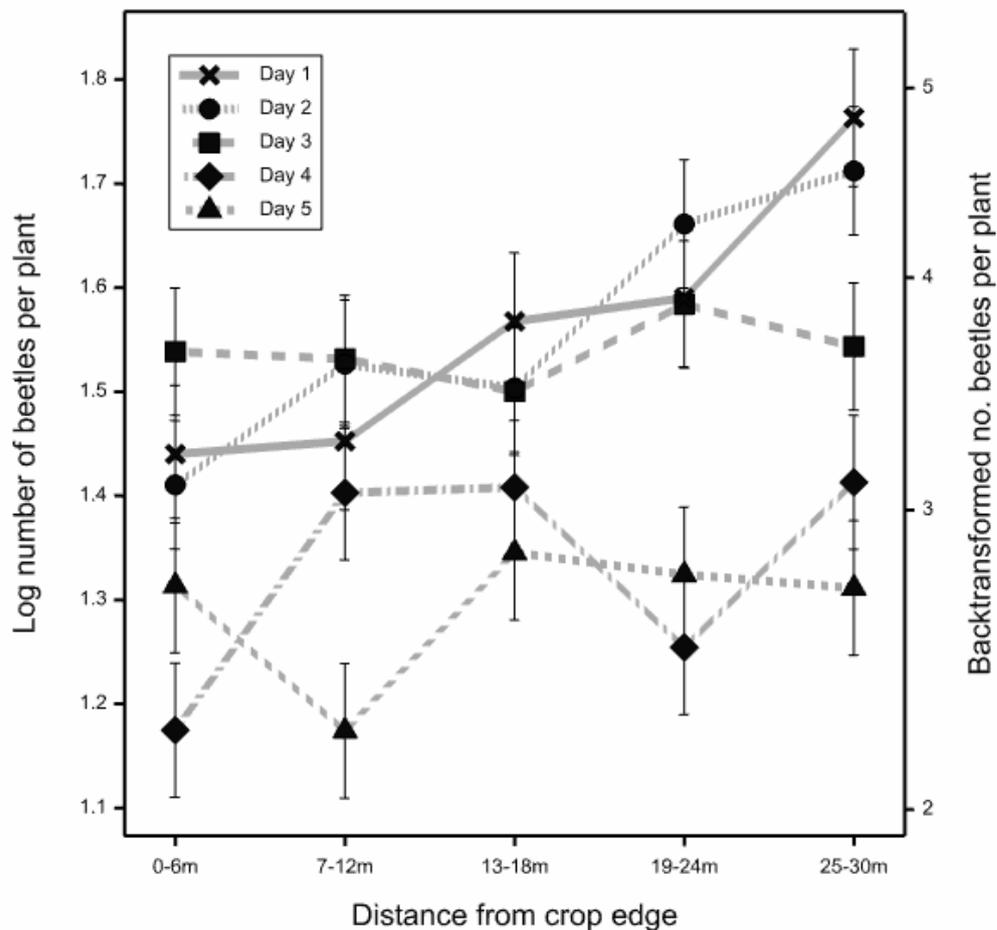


Figure 4. The log (x+1) transformed predicted mean ( $\pm$  e.s.e) number of pollen beetles per plant at each 6m segment along four transects, over five days towards the end of pollen beetle immigration.

### Conclusions and implications for monitoring

The mean number of pollen beetles differed between transects taken on different sides of the field. This varied with day and it is likely to be influenced by crop growth stage, meteorological effects and local landscape features including tree lines and hedgerows.

Monitoring populations of beetles in order to determine whether or not the action threshold has been breached along only one transect in one part of the field is therefore *not* sufficient to achieve reliable data for spray decisions.

Implications for the need to perform a transect into the field are less clear. The number of beetles per plant varied according to distance from the crop edge; with more beetles present in the crop centre than the border on days 1 and 2; indicating the need to perform monitoring along a transect to get an accurate estimation of population size. However, on days 3-5 the number of beetles per plant was more homogenous, indicating that transects are not necessarily needed for accurate population monitoring; plants can equally well be sampled nearer to the crop edge where it is easiest than along a long transect into the crop. It must be strongly emphasised that these data are derived from one field only, over a relatively short period. More data on a greater number of fields and over a wider period of immigration are required to deliver firm recommendations for best monitoring practice.

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# **Pathology papers**



## Potential effects of global warming on oilseed rape pathogens in northern Germany

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**Abstract:** The rise of mean air and hence soil temperature due to global warming will have effects on both crop and fungal pathogen development. Within the research framework KLIFF (Climate Change Research in Lower Saxony, Germany), potential effects of higher air and soil temperatures on the life cycle of the economically important oilseed rape pathogens *Leptosphaeria maculans*, *Sclerotinia sclerotiorum* and *Verticillium longisporum* are investigated both theoretically and experimentally.

Within the theoretical approach, published knowledge about temperature influences on certain life cycle stages of the pathogens, such as survival, sporulation, infection and further disease development, was compared with current climate change scenarios for the periods 2001-2030 and 2071-2100 at three different oilseed rape growing regions in northern Germany. There is evidence that warming might favour all three fungal diseases, but shifts in future prevalence of these pathogens may occur, favouring *Sclerotinia sclerotiorum* and *Verticillium longisporum* in particular.

In order to study effects of rising soil temperatures on the soil- and debris-borne life cycle stages of the three pathogens under field conditions, a soil warming experiment was established. Investigations include (1) ascospore release of *Leptosphaeria maculans* in autumn as well as subsequent stem canker development, (2) apothecia production of *Sclerotinia sclerotiorum* in spring and (3) the infection of winter oilseed rape by *Verticillium longisporum*. First year experiences and results of this soil warming experiment will be presented, including warming effects on plant growth, microclimate and fungal pathogen development.

**Key words:** Climate change, *Sclerotinia sclerotiorum*, *Leptosphaeria maculans*, *Verticillium longisporum*

Most researchers agree that climate change is mainly associated with a rise of mean air temperatures, but also a higher climate variability (IPCC, 2007). These changes may result in shifting of agro-climatic zones and hence directly influence crop cultivation (Tiedemann, 1996). Furthermore, fungal pathogens are likely to be affected by climatic changes at the same time. In oilseed rape, an important crop in Germany, the most severe economic losses result from the pathogens *Sclerotinia sclerotiorum*, *Leptosphaeria maculans* and *Verticillium longisporum*.

Knowledge on climatic requirements of these fungal pathogens is available but is strongly fragmented. Hence, a literature study was carried out within the research framework KLIFF (Climate Change Research in Lower Saxony, Germany) collecting published knowledge about temperature influences on certain life cycle stages of the pathogens, such as survival, sporulation, infection and further disease development (Table 1). In a second step, these data were compared with current climate change scenarios derived from the regional climate model REMO for the periods 2001-2030 and 2071-2100 at three different oilseed rape growing regions in northern Germany (Siebold and Tiedemann, 2011).

Table 1: Available published knowledge on climatic effects on different life cycle stages of three important pathogens in oilseed rape and existing modelling approaches. (n) = number of studies considered, (✓) = comprehensive data available ( $T_{\min}$ ,  $T_{\text{opt}}$ ,  $T_{\max}$  and moisture effects), (?) = available data are insufficient, (X) = significant knowledge gaps, (P) = models available as decision support systems for fungicide application by farmers.

| Pathogen                                    | Life Cycle Stages |             |           |                  | Modelling approaches |
|---|-------------------|-------------|-----------|------------------|----------------------|
|   | Survival          | Sporulation | Infection | Disease progress |                      |
| <i>Sclerotinia sclerotiorum</i><br>(n = 97) | ✓                 | ✓           | ✓         | ✓                | P                    |
| <i>Leptosphaeria maculans</i><br>(n = 74)   | ✓                 | ✓           | ✓         | ✓                | P                    |
| <i>Verticillium longisporum</i><br>(n = 37) | ?                 | ?           | X         | ?                | X                    |

There is evidence that warming might favour all three fungal diseases, but shifts in future prevalence of these pathogens may occur. Warming may shift the monthly mean temperatures in spring closer to the optimum temperature range of *S. sclerotiorum*, particularly in the longer term (2071-2100). Enhancement of the infection window for this pathogen under future warming was already suggested previously (Tiedemann and Ulber, 2008; Evans *et al.*, 2009). *L. maculans*, on the other hand, is already in its optimum temperature range for initial developmental stages in autumn, such as pseudothecia maturation and ascospore production. However, plant infection and systemic growth from leaf lesions into the stem may be favoured by milder autumns and winters (Tiedemann and Ulber, 2008). Sufficient moisture, however, will remain a key factor for the occurrence of both diseases. The existing knowledge on *V. longisporum* epidemiology is scarce (Table 1). Nevertheless, the apparent temperature optimum above 20 °C for most life cycle stages suggests a beneficial effect of warming on oilseed rape colonization by *Verticillium*. Global warming will also increase soil temperature, at least in the upper layers where fungal inoculum is present (Zhang *et al.*, 2005), and may finally shift soil temperatures towards the biological optimum of the fungus.

In order to study effects of rising soil temperatures on the soil- and debris-borne life cycle stages of the three pathogens under field conditions, a soil warming experiment was established. Investigations include (1) Phoma leaf spot development in autumn as well as subsequent stem canker development, (2) apothecia production of *S. sclerotiorum* in spring and (3) the infection of winter oilseed rape by *V. longisporum*. Preliminary results from the first experimental year, supplemented by climate chamber investigations, support the assumptions that were drawn from the literature study.

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## Fungal diseases of sunflower in Turkey

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**Abstract:** Sunflower (*Helianthus annuus*) is one of the main crops for oilseeds in Turkey. The sunflower has a number of pathological problems, especially fungi. In this review, the fungus species determined in Turkey until now are summarized. According to the relevant literature, 19 fungus species belonging to 16 genera are reported in Turkey. The *Sclerotinia sclerotiorum*, *Plasmopara halstedii* and *Macrophomina phaseolina* are some of the most important pathogens of sunflower.

**Key words:** Fungi, *Helianthus annuus*, Turkey

### Introduction

Sunflower (*Helianthus annuus* L.) belongs to the family Asteraceae. Sunflower is one of the important oilseed crops in both Turkey and worldwide. It is responsible for 44.5% of the total oil seed production in Turkey. According to the data of 2010, the average oilseeds production in Turkey was: rapeseed – 106,450 tons (31,250 ha); sunflower – 1,320,000 tons (641,400 ha); soybeans – 86,540 tons (23,473 ha); groundnut – 97,310 tons (27,450 ha); sesame – 23,460 (31,824 ha); safflower – 26,000 (13,500 ha); poppy seed – 36,910 tons; and cotton seed – 1,272,800 tons (Anonymous, 2011).

Sunflower oil is the most preferred vegetable oil in Turkey. Sunflower is also important in the animal and human food industry, cellulose industry, paint and soap industry and in medicine. High yields of quality sunflower require vigorous growth and healthy plants. Low yield of sunflower may be attributed to several reasons such as occasional adverse climatic conditions, poor agronomic methods of cultivation, non-availability of improved seed and prevalence of diseases and damage caused by pests. Sunflower like other crop suffers from many fungal diseases. More than 300 fungi species on sunflower have been reported worldwide (Farr & Rossman, 2011).

### Material and methods

Until now, no complete list of fungi species of sunflower in Turkey has been published. In this review, the fungi species determined in the literature are summarized.

### Results and discussion

In this paper, the former studies on the fungi species of sunflower in Turkey were summarized. According to the relevant literature, 19 fungus species belonging to 16 genera are reported in Turkey (Table 1).

Table 1. A list of fungi found on sunflower in Turkey.

| <b>Fungus species</b>                                       | <b>Localities</b>   | <b>References</b>  |
|---|---|--|
| <i>Alternaria</i> sp.,                                      | Balıkesir, Çanakkale, Edirne, İstanbul, Kırklareli, Tekirdağ  | Yücer & Karaca, 1978; Onan <i>et al.</i> , 1992  |
| <i>Alternaria alternata</i>                                 | Erzurum   | Demirci & Kordalı, 1998  |
| <i>A. helianthicola</i>                                     | Erzurum   | Demirci & Kordalı, 1998  |
| <i>Botrytis cinerea</i>                                     | Balıkesir, Edirne, Kırklareli,  | Özkutlu, 1976; Yücer & Karaca, 1978; Onan <i>et al.</i> , 1992   |
| <i>Cochliobolus nodulosus</i>                               | Erzurum   | Demirci & Kordalı, 1998  |
| <i>Colletotrichum atramentarium</i>                         | Erzurum   | Demirci & Kordalı, 1998  |
| <i>Erysiphe cichoracearum</i> var. <i>latispora</i>         |   | Braun, 1995  |
| <i>Fusarium acuminatum</i>                                  | Erzurum   | Demirci & Kordalı, 1998  |
| <i>F. arthrosporioides</i>                                  | Erzurum   | Demirci & Kordalı, 1998  |
| <i>F. equiseti</i>  | Erzurum   | Demirci & Kordalı, 1998  |
| <i>F. oxysporum</i>   | Erzurum, Tekirdağ   | Özer & Soran, 1994; Demirci & Kordalı, 1998  |
| <i>Helminthosporium</i> sp.                                 | Edirne, Tekirdağ  | Yücer & Karaca, 1978   |
| <i>Macrophomina phaseolina</i>                              | Balıkesir, Çanakkale, Erzurum, Eskişehir, Tekirdağ  | Onan <i>et al.</i> , 1992; Özer & Soran, 1994; Demirci & Kordalı, 1998; Mahmoud & Budak, 2011  |
| <i>Plasmopara halstedii</i> ( <i>Plasmopara helianthi</i> ) | Adana, Adapazarı, Ankara, Balıkesir, Çanakkale, Edirne, İstanbul, İzmir, Kırklareli, Samsun, Tekirdağ | Karel, 1958; Özkutlu, 1976; Yücer & Karaca, 1978; Çınar & Biçici, 1982; Döken, 1982; Karasu, 1982; Maden, 1982; Onan & Karcıoğlu, 1982, 1989; Özkutlu <i>et al.</i> , 1982; Delen <i>et al.</i> , 1985; Onan <i>et al.</i> , 1992; Demirci & Kordalı, 1998 |
| <i>Puccinia helianthi</i>                                   | Balıkesir, Çanakkale, Edirne, Erzurum, İstanbul, Kırklareli, Tekirdağ                                 | Yücer & Karaca, 1978; Onan <i>et al.</i> , 1992; Demirci & Kordalı, 1998   |
| <i>Pythium</i> sp.  | Erzurum   | Demirci & Kordalı, 1998  |
| <i>Pythium butleri</i>                                      | Adana   | Çınar & Biçici, 1982   |
| <i>Rhizopus</i> sp.   | Balıkesir, Çanakkale, Edirne  | Yücer & Karaca, 1978; Onan <i>et al.</i> , 1992; Özgen <i>et al.</i> , 2005  |
| <i>Rhizopus stolonifer</i>                                  | Adana, Çanakkale  | Çınar & Biçici, 1982; Yıldırım & Kaya, 2005; Yıldırım <i>et al.</i> , 2010   |
| <i>Sclerotinia minor</i>                                    | Edirne, Erzurum   | Çetinkaya & Yıldız, 1988; Demirci & Kordalı, 1998; Tozlu & Demirci, 2008   |
| <i>Sclerotinia sclerotiorum</i>                             | Adana, Balıkesir, Çanakkale, Edirne, Erzurum, Kırklareli, Tekirdağ                                    | Özkutlu, 1976; Yücer & Karaca, 1978; Yücer, 1980; Çınar & Biçici, 1982; Çetinkaya & Yıldız, 1988; Onan <i>et al.</i> , 1992; Demirci & Kordalı, 1998; Tozlu & Demirci, 2008  |
| <i>Septoria</i> sp.   | Edirne, Kırklareli,   | Yücer & Karaca, 1978   |
| <i>Ulocladium atrum</i>                                     | Erzurum   | Demirci & Kordalı, 1998  |
| <i>Verticillium dahliae</i>                                 | Balıkesir, Çanakkale, Erzurum   | Onan <i>et al.</i> , 1992; Demirci & Kordalı, 1998   |

Sunflower plants are attacked by a number of infection microorganisms mostly fungi, bacteria, virus and nematodes which reduces yield and quality. The most serious diseases of sunflower are caused by fungi. Downy mildew (*Plasmopara halstedii*), *Sclerotinia* stalk and head rot (*Sclerotinia sclerotiorum*) and charcoal rot (*Macrophomina phaseolina*) were reported as most destructive on sunflower crop in Turkey (Yücer & Karaca, 1978; Çınar and Biçici, 1982; Döken, 1982; Onan et al., 1992; Demirci & Kordalı, 1998; Tozlu & Demirci, 2008; Özer & Soran, 1994; Mahmoud & Budak, 2011).

Several other fungal pathogens also attack the sunflower including *Alternaria*, *Botrytis*, *Cochliobolus*, *Colletotrichum*, *Erysiphe*, *Fusarium*, *Helminthosporium*, *Puccinia*, *Pythium*, *Rhizopus*, *Septoria*, *Ulocladium* and *Verticillium* (Table 1). These fungi can attack the roots, stems, leaves, or heads, but only a few of these diseases cause significant economic loss. To control of disease, The Ministry of Agriculture and Rural Affairs technical instructions should be used.

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## **WIN: Developing site-specific advisories for agricultural producers**

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**Abstract:** Weather INnovations Incorporated, (WIN) is a research spin off from the University of Guelph in Ontario, Canada which began commercial operation in 2007. WIN specialises in providing turnkey weather based monitoring and modelling solutions for agribusinesses, producer organisations, government agencies, researchers and others. The primary goal of WIN is to help facilitate the delivery of science based decision support systems to agricultural producers who need straightforward advisories and tools which are easily accessible on the web. As a private company, WIN is completely independent and offers a flexible, service minded solution for a number of producer groups and multinational clients. As part of this service, WIN maintains and manages >1600 weather stations, mainly in North America, but has recently been deploying monitoring equipment in agricultural landscapes in Europe. WIN offers an ever increasing number of online Decision Support System products for a range of crops, based on real-time weather data. These include BEETcast (cercospora leaf spot on sugar beet), TOMcast (anthracnose, septoria and blights on tomato), SPUDcast (late and early blight of potato), DONcast (predicting DON [deoxynivalenol toxin] levels at heading in wheat) WHEATcast (septoria and powdery mildew on wheat) and SPRAYcast (forecast of spraying conditions to minimize spray drift; Figure 1). WIN creates and manages websites for its clients so information can be well targeted to the client's needs (for example [www.vineandtreefruitinnovations.com](http://www.vineandtreefruitinnovations.com)). Recently, many of the websites have been further developed for mobile devices (Figure 2). Further, WIN operates custom monitoring programs for crop insurance and irrigation infrastructure management. WIN ([www.weatherinnovations.com](http://www.weatherinnovations.com)) is currently working to provide similar online solutions to other pests including pests that affect canola in Canada and oilseed rape in Europe.

**Key words:** Decision support system, modelling, monitoring, arable crops, canola, fungicide treatment

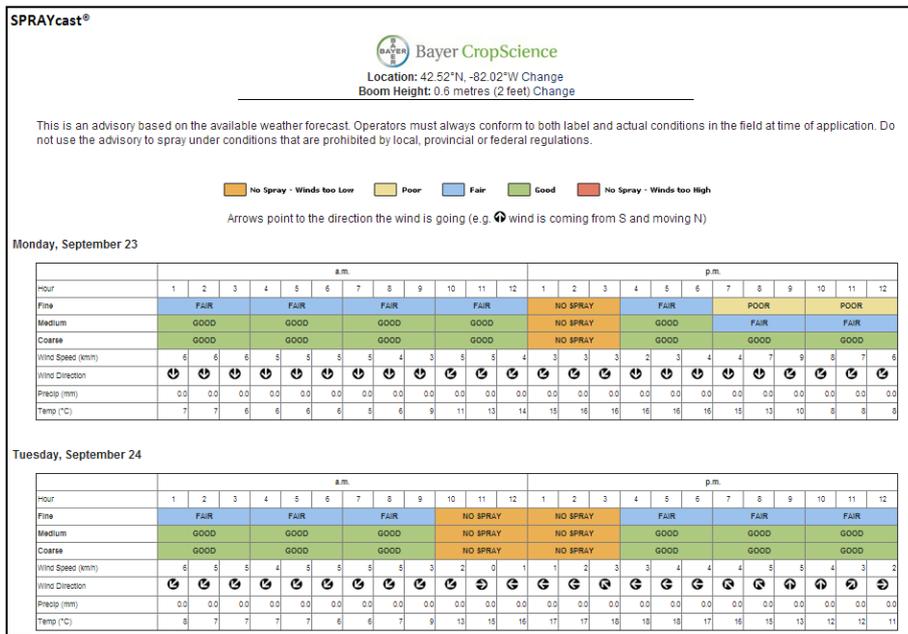


Figure 1. SPRAYcast® uses decision support criteria, developed by Environment Canada, incorporated in a 3 day weather forecast (Days 1 and 2 shown here). Growers can choose their field location, to obtain weather forecast data pertinent to their field operation.



Figure 2. Newly developed „mobile“ websites configured specifically for hand-held devices such as mobile cellular phones and tablets. The example shown here is [www.vineandtreefruitinnovations.com](http://www.vineandtreefruitinnovations.com) developed for the grape and soft fruit growers of Ontario.

## **KILA – the new project on clubroot and stem canker of oilseed rape in Poland**

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**Abstract:** The new project on clubroot and stem canker of winter oilseed rape commenced in Poland in autumn 2010. The project will last for three years and is funded by the Polish Ministry of Science and Higher Education. The main goal of the project is the search for resistance sources to clubroot and stem canker in selected mutants of *Brassica napus* and interspecific hybrids among the genus *Brassica*. Stem canker, caused by *Leptosphaeria maculans*, and clubroot, caused by *Plasmodiophora brassicae*, are diseases that greatly reduce the yield of oilseed rape in Poland; the former disease is well established since the 1980's, whereas the latter is a new disease, fast expanding in all regions of intensive cultivation of oilseed rape. The research aims to determine the composition of the population of *P. brassicae*, which is not known in Poland at all, and to monitor further *L. maculans*; comparing its current population with previous findings and to identify newly emerging pathotypes or races. So far, over 50 samples of *P. brassicae* have been collected from numerous geographic regions of Poland. These demonstrate that clubroot is not only present, but ubiquitous. The most prevailing races of the pathogen will be used to study the resistance of hybrids of *B. napus* x *B. rapa* and *B. napus* x *B. juncea* as well as 32 mutants of *B. napus* with increased tocopherols and decreased amounts of indole glucosinolates. We plan to check the usefulness of selected molecular markers and study which genomes and chromosomes contain the detected resistance to diseases, using GISH and FISH techniques.

**Key words:** *Brassica* sp.; interspecific hybrid; resistance; *Plasmodiophora brassicae*, *Leptosphaeria maculans*



# **Biocontrol**



## Biocontrol of *Sclerotinia* stem rot – cornerstone in durable high-intensity rape production

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**Abstract:** Rapeseed production in the EU-15 has doubled in the past 20 years reaching at present more than 15 million tons while the area of cultivation has increased by about 65%. The underlying intensification of rapeseed production mainly consisted of a narrowing of crop rotations which has enhanced disease problems and increased the number of fungicide applications. Nonetheless, chemical control of diseases like *Sclerotinia* stem rot has not been able to counteract the overall increase of disease pressure, since this pathogen forms long-lasting inoculum, sclerotia, which may survive in the soil for at least 12 years and is not controlled by fungicides. Moreover, *Sclerotinia sclerotiorum*, besides stem rot, has induced additional disease types like root rot and young plant damage, recently. Since no effective control through resistant cultivars is available, current control does not appear durable under high-intensity conditions of rapeseed cultivation. A potential improvement is post-harvest treatment of rapeseed plant debris with the conidia of the antagonistic mycoparasite *Coniothyrium minitans*. *C. minitans* attacks the sclerotia and penetrates the sclerotial rind and medulla, whereafter pycnidia are formed in the host tissue. Thereby, *C. minitans* propagates and may establish and survive for several years in the field soil. *C. minitans* has been shown to be superior to any other sclerotial colonizers having a mycoparasitic potential. A more recent finding is, that *Microsphaeropsis ochracea*, known as a potent degrader of melanised fungal structures of *Venturia inaequalis*, *Gibberella zeae* and *Rhizoctonia solani*, has a high potential to degrade sclerotia of *S. sclerotiorum*. In a lab assay, *M. ochracea* at an inoculum density of  $10^5$  conidia/ml, induced 100% mortality of sclerotia within 3 weeks. Fluorescence microscopic studies under the confocal laser scanning microscope with reporter gene transformed (DsRed, GFP) transgenic strains of *M. ochracea* and *C. minitans* demonstrated the ability of *M. ochracea* to invade and colonize vital sclerotia, finally resulting in internal formation of pycnidia and complete sclerotial degradation. In a direct comparison with *C. minitans*, mycoparasitic activity of *M. ochracea* was lower and colonization pattern more restricted to the periphery of sclerotia. Treatment of sclerotia with a mixture of *C. minitans* and *M. ochracea* did not increase the overall mycoparasitic potential. The effect of *C. minitans* was particularly superior at lower inoculum densities ( $< 10^4$  conidia/ml).

**Key words:** *Sclerotinia sclerotiorum*; *Coniothyrium minitans*; *Microsphaeropsis ochracea*; DsRed; GFP

## **Studies on *Trichoderma* in protection of winter oilseed rape against fungal diseases**

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**Abstract:** According to the newest directives of the European Community, the management of pests and diseases shall combine integrative practices that are both effective and environmentally friendly. The amount of pests should be reduced using the most economical ways with the least possible hazard to people and the environment. In recent years there has been increasing interest in antagonistic and hyperparasitic fungi, which have potential in combating plant pathogens and thus reducing the amount of pesticides needed in crop protection. One of the most powerful biocontrol agents for use against plant pathogens, are the representatives of the genus *Trichoderma*. The aim of this work was to check the potential of the genus *Trichoderma* to control fungi pathogenic to oilseed rape (*Brassica napus*). The fungal materials used in the study comprised the isolates belonging to five species, including *T. atroviride*, *T. hamatum*, *T. harzianum*, *T. koningii* and *T. longibrachiatum*. Isolates of these fungi were obtained from oilseed rape and yellow lupin plants as well as from the soil from rapeseed fields. The studies were performed under laboratory and field conditions. The laboratory experiments were done on agar media using dual culture bioassays with most important fungal pathogens growing side by side with the cultures of *Trichoderma*. The studies were done using 19 isolates, belonging to the following species: *Alternaria alternata*, *A. brassicae*, *A. brassicicola*, *Botrytis cinerea*, *Leptosphaeria biglobosa*, *L. maculans*, *Sclerotinia sclerotiorum* and *Verticillium longisporum*. The cellulolytic activity of the isolates was also studied using Filter Paper Assay. The level of absorbance was measured at  $\lambda = 530$  nm. The cellulolytic activity was measured in Filter Paper Units (FPU), where 1 FPU = 1  $\mu\text{mol/ml} \times \text{min}$ . In field experiments the plants of two cultivars of WOSR were treated with four doses of spore suspension in two periods during the autumn, and in three consecutive seasons from 2008/2009 to 2010/2011. The collected stubble was also sprayed with spore suspensions of five *Trichoderma* species. It was found that in dual cultures the tested isolates of *Trichoderma* sp. efficiently controlled the pathogens of oilseed rape. They decreased the growth of the pathogens on agar cultures, especially of *Verticillium longisporum* and *Leptosphaeria biglobosa*. The species of *Trichoderma* differed in their hyperparasitic properties, mainly towards *Leptosphaeria maculans*, where an antibiosis effect was observed in dual cultures with *T. longibrachiatum* and *T. harzianum*. The highest cellulolytic activity was found for the isolates of *T. harzianum*. In the proces of the hydrolysis of cellulose the most efficient isolate was *T. koningii*. This isolate was also the slowest in growth and the least abundant in sporulation. The highest retardation of growth and development was observed for *L. biglobosa*. In contrast, *L. maculans* slowed down the growth of *Trichoderma* spp., especially of *T. longibrachiatum*. The *L. maculans* phytotoxin sirodesmin PL has been added to liquid cultures of several *Trichoderma* isolates to study its role in this inhibition. No significant decrease or decomposition of sirodesmin PL has been observed in these cultures so far. Field studies have demonstrated a very weak effect of *Trichoderma* spp. on phoma leaf spotting of WOSR. The trend was positive, but low or no statistical correlations were found. The spore suspension of *Trichoderma* sprayed on stubble of WOSR decreased the amount of crude fiber by 7.4% (after two weeks) to 16.7% (after two months).

**Key words:** *Brassica napus*; biological control; *Verticillium longisporum*; *Leptosphaeria maculans*; *Leptosphaeria biglobosa*

## **Blackleg, stem canker**



## Forecasting system for blackleg (*Leptosphaeria maculans* and *Leptosphaeria biglobosa*) of crucifers in the Czech Republic

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**Abstract:** Blackleg is a serious global disease of crucifers. It is caused by two pathogens *Leptosphaeria maculans* and *L. biglobosa*. In the Czech Republic, canola and oilseed rape are attacked each season and yield losses can be 20%. To help growers protect their crops from blackleg attack, prediction systems are used to help target fungicide applications in some other countries in Europe (Poland, UK). In 2008, the Czech Ministry of Agriculture commissioned a project (QH81127) to develop a forecasting system to help oilseed rape growers and agricultural chemical company representatives to recognize when crops were at risk from high levels of infection with the aim of reducing yield losses. Five Czech research institutions worked on the project in collaboration with DuPont who provided meteorological data (precipitation, temperature) for input to the system which allowed us to predict the risk of infection from blackleg. At five different sites (Šumperk, Opava, Prague, Brno and Kroměříž) we monitored for the presence of airborne ascospores of *Leptosphaeria* spp. in both spring and autumn. These data, along with associated meteorological data, allowed us to forecast high risk periods in order to advise growers of the need for preventive or curative treatments on oilseed rape crops. In 2009/10, a dedicated website was also made available to provide information about the risk of infection.

**Key words:** Phoma stem canker; forecast; ascospore release; meteorological data; fungicide treatment



## Identification of Phoma risk years and regions with the decision support system proPlant expert

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**Abstract:** The proPlant expert crop protection consultation system helps farmers and advisers with their decisions regarding control of Phoma leaf spot and the use of growth regulators in autumn.

The first question the system answers is, if the current weather promotes Phoma leaf spot infections. Taking into account the previous days and a three-day-forecast, the system finds out whether recent weather conditions were conducive or become conducive to Phoma infections in the following days. In parallel, the system also analyses the increase of Phoma risk since crop emergence.

The second question proPlant expert answers is, if a fungicide application against Phoma leaf is required this autumn, when are the optimal dates and what needs to be considered regarding growth regulator use. The field-specific recommendation considers both, the need of Phoma leaf spot treatment and the need of growth regulator use (timing, choice of fungicide).

The use of fungicides against Phoma leaf spot in autumn makes sense only if several leaves have already developed, if Phoma leaf spot infestation is visible and if the weather during the previous days promoted new infections. In this case a curative treatment leads to a high degree of efficiency (infection-based fungicide strategy). The system advises against a fungicide application if no infestation is visible yet or if infestation cannot develop because of the weather during the previous days. Depending on the year and the region, the optimal date for treatment in Germany can be as early as the 20<sup>th</sup> of September or after mid of October.

proPlant expert analyses the weather data and the user gives the information to the system if infestation is visible in the field or not. From these and further data (e.g. susceptibility of the variety, growth stage) proPlant expert derives a concrete decision for treatment with a selection of suitable fungicides and application rates. The requirements regarding growth regulation and frost resistance are also taken into account.

The third question “how do fungicides differ in the efficacy against Phoma leaf spot” is also answered by proPlant expert, because it contains a database with curative and protective efficacy (given in degree days) of registered Phoma leaf spot fungicides.

**Key words:** oilseed rape; *Leptosphaeria maculans*; *Leptosphaeria biglobosa*; fungicide treatment; growth regulator; timing; infection days; weather data

The computer-based crop protection consultation system “proPlant expert” enables users to identify years and regions with high risk of early Phoma leaf spotting (*Phoma lingam*) on winter oilseed rape. Farmers and advisers get comprehensive field-specific decision support on Phoma control and growth regulation in autumn since the year 2006.

In Germany, the acreage of winter oilseed rape increased continuously in recent years and where oilseed rape forms a high proportion of arable cropping, the risk of Phoma is high. New oilseed rape crops are close to fields with previous year’s oilseed rape stubble, the sources of new Phoma infections. Oilseed prices increased and are still at a high level. This generally justifies higher chemical input. New fungicides for autumn use have been registered – some are more effective as plant growth regulator, others more for Phoma control. Because of the extended selection of fungicides, farmers can choose the appropriate product and dosage depending on the situation.

Phoma is widely spread in Germany but with seasonal and regional variation in disease incidence. The onset of leaf spotting and the infestation rates are very variable. Fungicide applications in autumn against Phoma are not always profitable. In many years additional yield does not cover the treatment costs. But on the other hand it is known that early Phoma leaf spotting has usually more impact on the yield than infections in spring, because they can lead to early cankers. In years with early presence of disease in autumn significant additional yields are possible with fungicide treatments. And in addition also requirements of plant growth regulation for better winter hardiness need to be considered.

The proPlant expert system identifies the risk of early Phoma epidemic mainly based on weather data. Dry conditions from emergence until mid-October do not encourage Phoma infections and early Phoma leaf spot attack (e.g. 2009 in Germany). Consequently, proPlant Phoma risk prognosis is green. Fungicide use should focus on the growth regulation needs. In Germany winter oilseed rape is sown from the beginning of August until the beginning of September, usually from 15<sup>th</sup> until 25<sup>th</sup> of August. Early sown crops that developed 4-6 leaves by mid-September need early treatment to avoid overgrowing.

Wet conditions in August and September however promote spreading of the disease and can lead to an early start to the Phoma leaf spot phase. Phoma requires rainfall. Temperatures are usually not limiting for disease development at that time of the year.

If proPlant indicates many days with optimal conditions for Phoma infections since crop emergence, proPlant prognosis switches from green to yellow by the end of August (Figure 1). Crops should be monitored regularly from then on, because Phoma leaf spot symptoms are likely to become visible. If the conditions for Phoma infections stay optimal, the risk of severe epidemics increases quickly and proPlant prognosis turns to red by mid-September. This was the case 2010 in Germany, especially in the Northern parts of the country with an early and severe leaf spot attack (Figure 2). In years like this, the date of fungicide application, the product choice and dosage depended on the required activity against Phoma.

To get the spray timing right for control of Phoma is not simple, but necessary for significant additional yields. proPlant advises fungicide treatment if

- Phoma leaf spot symptoms have started to appear,
- plants have developed at least 4 leaves,
- weather was favourable for Phoma infections during the previous days.

proPlant recommends to apply fungicides with curative (kick-back) activity against Phoma shortly after infections. This “infection-based strategy” usually leads to a high degree of Phoma control efficacy. The system advises against a fungicide application if no leaf spots appeared in the crop yet or if the weather during the previous days did not promote Phoma infections (no rainfall). Depending on the year and on the region the optimal date for Phoma leaf spot treatment in Germany can be already mid-September or not before mid-October (Table 1).

proPlant expert also provides a comprehensive field-specific consultation, which assists farmers in making decisions on the need of Phoma leaf spot control and growth regulator use in autumn. The consultation is based not only on weather data but also on crop data given to the system by the user, e.g. variety, growth stage, crop vigour and Phoma infection level. From all these data the system derives a concrete advice for fungicide treatment with recommendation of suitable products and application rates (Figure 3). The fungicide suggestion is based on a database on the curative (kick-back) and preventive activity against Phoma leaf spot and on the growth regulating effect. The assessment of the fungicide performance is done by proPlant GmbH. It is impartial and based on the results of official field trials. The database is updated annually. If no Phoma leaf spots are seen, proPlant

recommends fungicides only according to the requirements of growth regulating and winter hardiness.

Various organisations and companies provide proPlant Phoma prognosis services for their customers: for example, the oilseed rape breeding association Rapool runs a Phoma warning service on its web pages for free ([www.rapool.de](http://www.rapool.de)). It includes amongst others nationwide and daily updated maps showing the current Phoma risk depending on weather based infection probabilities, local cropping density and previous year's infestation levels (see: Ep-01 PC demonstration: The proPlant expert decision-support system for pest and disease management in oilseed rape).

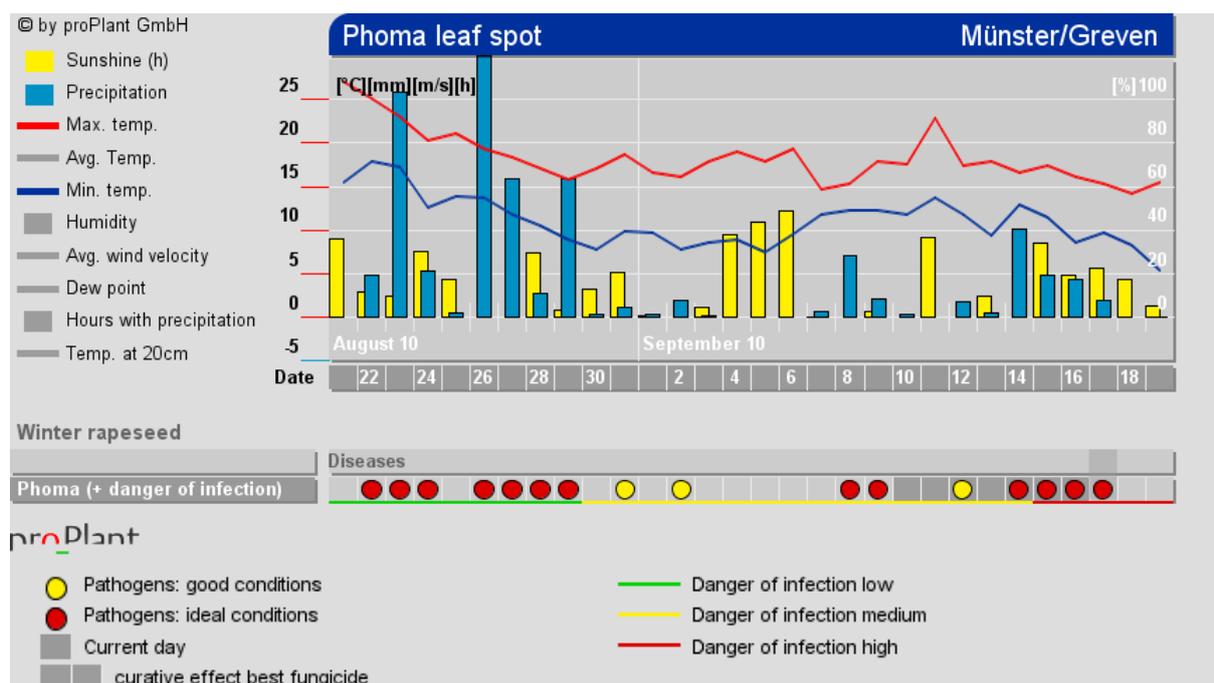


Figure 1. The proPlant expert Phoma infection graph covers the previous weeks and a three-day weather forecast. Red or yellow dots mark days with optimal resp. good weather conditions for Phoma infections. The accumulated danger of infection since crop emergence is given in the graph as risk line. It forecasts the risk of early start of leaf spot phase. Because in 2010 rainfall end of August and mid of September encouraged Phoma infections on many days, the colour of the risk line switched from green to yellow already end of August and from yellow to red mid of September, warning against the early Phoma epidemic that year.

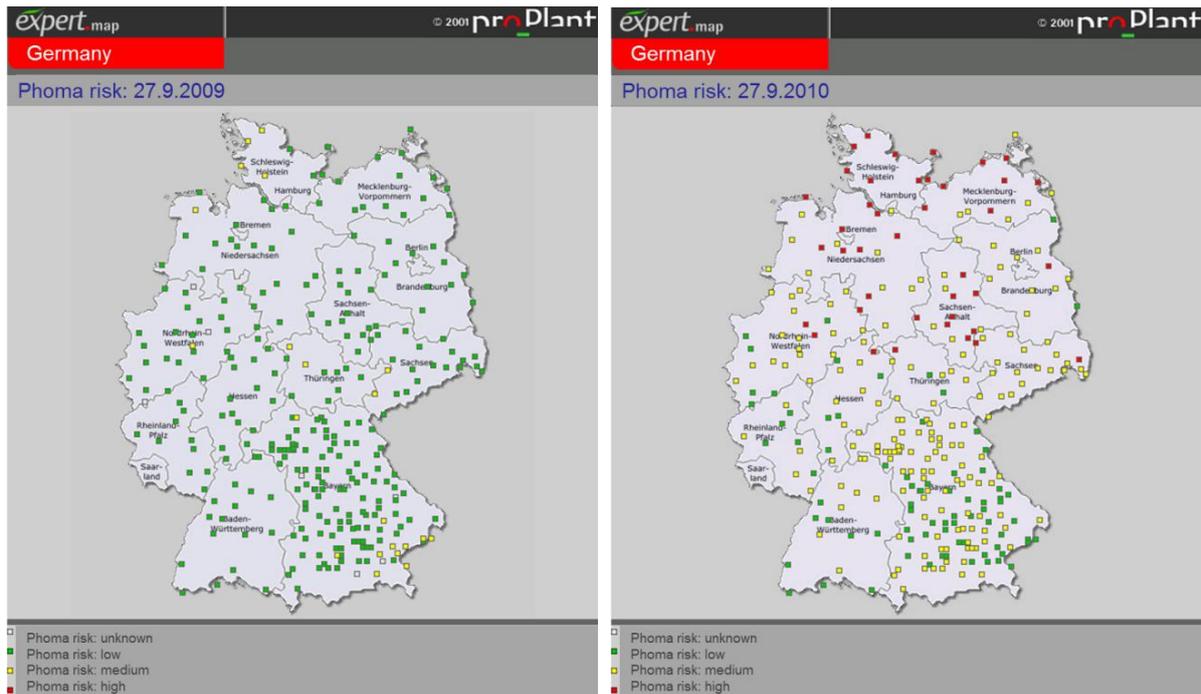


Figure 2. For a quick overview the Phoma risk forecasted by proPlant is shown in maps: End of September 2009 Phoma risk was low in most regions of Germany. Because the weather did not promote leaf spot attack since crop emergence, for most weather stations proPlant Phoma risk prognosis was still green. End of September 2010 however weather based Phoma risk was medium till high in the northern parts of Germany (proPlant prognosis for many weather stations already red), indicating the early start of leaf spot phase.

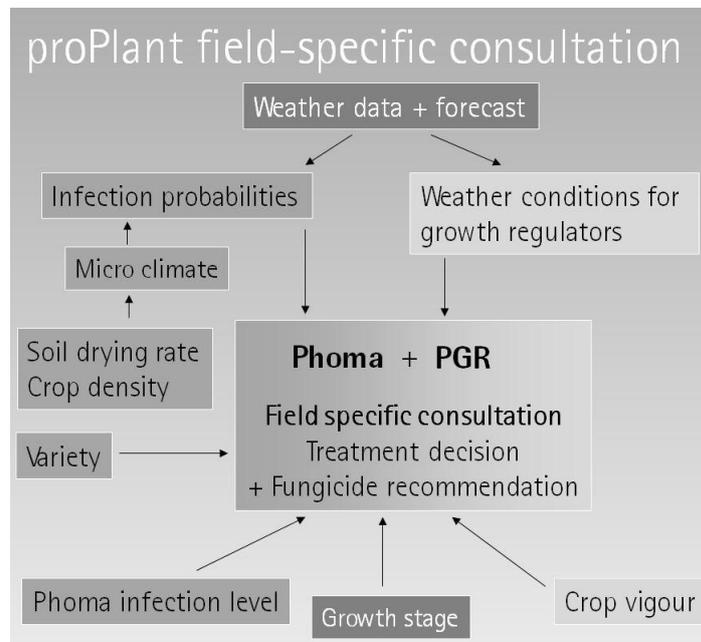


Figure 3. The field-specific consultation of proPlant expert decision support system in autumn aims both, effective Phoma leaf spot control and optimal use of plant growth regulators (PGR).

Table 1. proPlant decision support for use of fungicides and plant growth regulators in autumn depending on the weather-based Phoma risk

| <b>proPlant<br/>Phoma risk<br/>prognosis</b> | <b>Situation</b>  | <b>Main focus<br/>of treatment</b>                  |
|--|---|---|
| GREEN  | The risk of early Phoma leaf spot attack is low, because the weather was not favourable for Phoma infections. Please use the service again in the next few days. Current applications should orient to growth regulation needs.   | Growth regulation with side-effect on Phoma         |
| YELLOW                                       | The risk of early Phoma leaf spot attack is medium. First leaf spots can become visible in the field. Start field inspections now. An application is recommended if leaf spot symptoms appeared, plants have at least 4 leaves and if the weather during the previous days was favourable for Phoma infections. The treatment can be delayed if the current weather is not favourable for Phoma infections or if no leaf spots are visible yet. | Growth regulation with side-effect on Phoma         |
| RED  | The risk of early Phoma leaf spot attack is high. Field inspections are essential. An application is recommended if leaf spot symptoms appeared, plants have at least 4 leaves and if the weather during the previous days was favourable for Phoma infections. The treatment can be delayed if the current weather is not favourable for Phoma infections or if no leaf spots are visible yet.   | Phoma control with side-effect on growth regulation |



## Eight years' experience of the SPEC forecasting system for oilseed rape protection in Poland

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**Abstract:** Numerous pathogens of plants are broadcasted by air, which allows them to be dispersed over very large areas. Unlike soil pathogens that are persistent in certain areas or fields, and are transferred to new sites mainly on agricultural machinery or sometimes via seeds or tubers, airborne pathogens can easily move over tens- or even hundreds of kilometers. This allows them to find new ecological niches suitable for growth and development. Pathogen dispersal is the key point of each infection process and may greatly vary between species, regions and host-plants and is affected by the weather and regional microclimates. Aerobiology offers tools and methodologies allowing monitoring of airborne inocula. This process allows us not only to gather theoretical knowledge on pathogens' development but also provides valuable information for agricultural practice and can identify the crucial periods for plant protection with fungicides.

In Poland a network of 10 volumetric spore samplers located in different geographical locations has been constantly operating since autumn 2004. The monitoring is known as the System for Forecasting Disease Epidemics (*in Polish*: System Prognozowania Epidemii Chorob, SPEC). From the very start, the monitoring of airborne ascospores has been focused on *Leptosphaeria maculans* and *L. biglobosa*, two pathogens of oilseed rape, responsible for economic losses due to blackleg or stem canker. The communications about the concentration of the inoculum in air samples are immediately passed to farmers, using a website and SMS text messages sent to registered users. The data distribution in real-time is possible due to these technologies of message delivery. In Poland the communications of the SPEC decision support system are being sent using mobile nets to 3000 registered users, four times per season. The educational website ([www.spec.edu.pl](http://www.spec.edu.pl)) offering scientific descriptions is visited by *ca.* 4000 website users per year and the commercial website ([www.dupont.pl](http://www.dupont.pl)) offering a complex information service as well as advice is visited by nearly 10,000 users each year. The numbers show high interest in the use of aerobiological data in helping to undertake decisions in plant protection against the most serious diseases of agricultural crops. The methods can be easily implemented to other pathogens and geographical regions, for example as already demonstrated in north Poland, for the inoculum of *Pyrenopeziza brassicae*, the cause of light leaf spot. Studies on the dispersal of inoculum of other fungal pathogens in oilseed rape, cereals and other crops are currently under investigation.

**Key words:** *Brassica napus*; stem canker; *Leptosphaeria maculans*; *Leptosphaeria biglobosa*; spore trapping

## **Monitoring after the introduction of a new specific resistance against *Leptosphaeria maculans* in oilseed rape in a pilot production area**

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**Abstract:** Recently, new genotypes of oilseed rape were introduced commercially. Their excellent resistance to blackleg (*Leptosphaeria maculans*) is mainly due to a new major resistance gene Rlm7. Such varieties are potentially exposed to a resistance break down risk. We promote a specific monitoring in a production area where the risk seems to be particularly high. This area of around 100 km<sup>2</sup> is located in the central region of France between the towns of Issoudun and St Florent sur Cher. In this area 20 to 30 farmer's fields are observed each year. Rlm7 varieties were partially introduced in 2004, but were grown widely in the following years. Observations over the seasons include agronomic diagnosis, ascospores releases each autumn, and leaf spots sampling, both on Rlm7 and non Rlm7 cultivars. From sampled leaves, fungus isolates were characterized for virulence profiles. This paper presents the main results for seven successive cropping years and associated progress in detection methods.

**Key words:** *Brassica napus*; stem canker; Rlm7; virulence

## Investigating quantitative resistance to *Leptosphaeria maculans* (Phoma stem canker) in *Brassica napus* (oilseed rape) in controlled conditions

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**Abstract:** Operation of quantitative resistance to *Leptosphaeria maculans* in *Brassica napus* at the seedling stage was investigated in controlled environments using doubled haploid (DH) lines A30 (very susceptible, without quantitative resistance) and C119 (with good level of quantitative resistance). These two DH lines were derived from the cross Darmour-*bzh* × Yudal. Quantitative resistance to *L. maculans* at the seedling stage was investigated at two stages; growth of pathogen in leaf petioles before it reaches the stem and growth in stem tissues to form stem canker symptoms. Two types of inoculum (ascospores and conidia), two inoculation methods (leaf lamina inoculation and petiole inoculation) and different assessment methods (assess visible disease symptoms; trace symptomless pathogen growth using reporter gene GFP; quantify pathogen DNA) were used to study the quantitative resistance to *L. maculans* in controlled conditions. For leaf lamina inoculation, significant differences were observed between DH lines A30 and C119 in the rate of growth of GFP-expressing *L. maculans* and the amount of *L. maculans* DNA in leaf petioles. For leaf petiole inoculation, significant differences were observed between A30 and C119 in severity of stem canker and the amount of *L. maculans* DNA in stem tissues. These results indicate that it is possible to investigate quantitative resistance to *L. maculans* at the seedling stage.

**Key words:** ascospores, conidia, infection, quantitative resistance, oilseed rape, Phoma stem canker/blackleg

### Introduction

Phoma stem canker, caused by *Leptosphaeria maculans*, is the major disease problem on oilseed rape (*B. napus*) in Europe, currently causing UK annual losses worth more than £ 100M, despite use of fungicides costing £ 20M p.a. (Fitt *et al.*, 2006; Stonard *et al.*, 2010; Huang *et al.*, 2011). These losses will increase if the most effective fungicides are no longer permitted by EU legislation. Furthermore, it is predicted that global warming will continue to increase the range and severity of UK Phoma stem canker epidemics (Evans *et al.*, 2008). Host resistance has been the most economical and effective method for control of this disease. Two types of resistance to *L. maculans* in *B. napus* have been identified; qualitative (*R* gene-mediated) complete resistance expressed in leaves and quantitative partial resistance expressed in stems later (after initial leaf infection) (Delourme *et al.*, 2006; Huang *et al.*, 2009). Qualitative resistance to *L. maculans* is race-specific and is effective in protecting plants only if the corresponding avirulent allele is predominant in the local pathogen population (Balesdent *et al.*, 2001). Qualitative resistance usually loses its effectiveness

within three cropping seasons of widespread use in commercial cultivars because of selection for virulence within the variable *L. maculans* population. Quantitative resistance is considered to be race non-specific and more durable than qualitative resistance (Delourme *et al.*, 2006). However, quantitative resistance to *L. maculans* mainly operates during a long period (5-7 months) which includes the symptomless growth after appearance of leaf lesions in autumn and the development of visible cankers on stems in spring/summer. Selecting cultivars for quantitative resistance currently relies on field experiments in which stem canker is assessed on adult plants before harvest (Pilet *et al.*, 1998; Fitt *et al.*, 2006). Few methods have been proposed to assess quantitative resistance to *L. maculans* at the seedling stage in controlled conditions (Travadon *et al.*, 2009). Recently, petiole inoculation methods were used to compare either aggressiveness of different *L. maculans* isolates or quantitative resistance of oilseed rape genotypes on stem canker development at the seedling stage in controlled conditions (Brun *et al.*, 2011; Delourme *et al.*, 2011).

In the UK, epidemics of Phoma stem canker are initiated by air-borne *L. maculans* ascospores (West *et al.*, 1999; Huang *et al.*, 2005), which infect the leaves of winter oilseed rape in autumn (October/November), causing leaf lesions. From these leaf lesions, the pathogen grows without symptoms along the petiole to reach the stem where, in spring/summer (May-July), it causes stem cankers and resulting yield loss (Hammond *et al.*, 1985; Huang *et al.*, 2009). After initial infection of leaves (i.e. after appearance of Phoma leaf spots), the growth of *L. maculans* in oilseed rape can be considered in two stages; growth in leaf petioles before it reaches the stem and growth in stem tissues to form stem canker symptoms (Figure 1). This paper describes experiments using different inoculum and different inoculation methods to investigate the growth of *L. maculans* in leaf petioles and stem tissues of two oilseed rape doubled haploid (DH) lines differing in quantitative resistance.

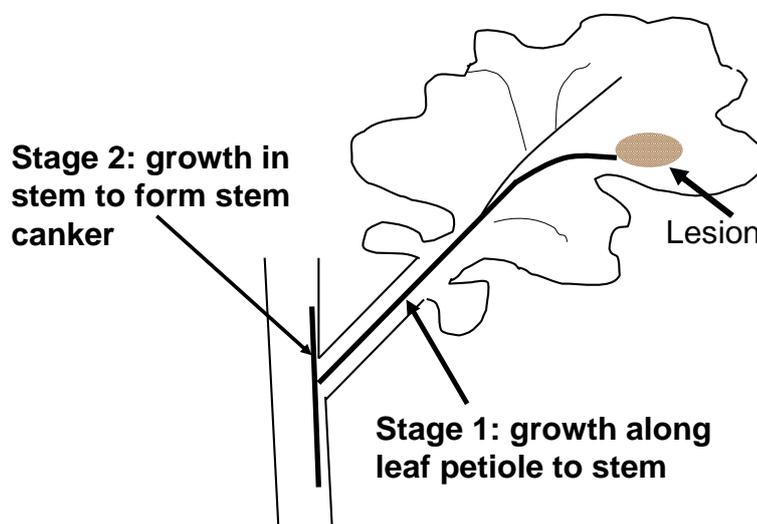


Figure 1. Two stages of *L. maculans* growth in oilseed rape used to investigate quantitative resistance to *L. maculans*

## Material and methods

### *Preparation of plant material and inoculum*

Plants of DH lines A30 or C119 derived from Darmour-*bzh* × Yudal population (Pilet *et al.*, 1998) were grown in pots (9 cm diameter) containing peat-based compost and a soluble fertiliser. For leaf lamina inoculation, experiments were arranged as randomised block design with two blocks. In each block, eight plants of each DH line were used for GFP-expressing conidia inoculation; 5 plants of each DH line were used for ascospore inoculation. For leaf petiole inoculation, experiment was arranged as complete randomised design with 5 plants of each DH line. Plants were initially grown in a glasshouse (20–23 °C) and thinned to one plant per pot 10 days after sowing. Three weeks after sowing when each plant had three expanded leaves, the plants were transferred to a growth cabinet (20 °C day/20 °C night, 12 h light/12 h darkness, light density 210  $\mu\text{e m}^{-2}\text{s}^{-1}$ ) overnight before inoculation.

Conidial suspensions of *L. maculans* isolate ME24 and GFP-expressing isolate ME24/3.13 were prepared from 12-day-old cultures on V8 agar as described by Huang *et al.* (2006). A suspension of *L. maculans* ascospores was prepared from naturally infected UK oilseed rape stem base debris collected in August 2007 using the method described by Huang *et al.* (2003). The concentration of conidia was adjusted to  $10^7$  conidia  $\text{ml}^{-1}$  and the concentration of ascospores was adjusted to  $10^4$  ascospores  $\text{ml}^{-1}$ , using a haemocytometer slide.

### *Plant inoculation*

For leaf lamina inoculation with GFP-expressing conidia of isolate ME24/3.13, the lower part of the leaf lamina was gently rubbed using an eraser and wounded using a sterile pin, then a 10  $\mu\text{l}$  droplet of conidial suspension was placed on each wound. Each leaf had two inoculation sites, one on each side of the main vein. For leaf inoculation with ascospores, the lower part of the leaf lamina was gently rubbed using an eraser without wounding and a 15  $\mu\text{l}$  droplet of ascospore suspension was placed on each rubbed site. Each leaf had two inoculation sites, one on each side of the main vein. For leaf petiole inoculation, the lower part of the leaf petiole (about 1 cm to the stem) was wounded using a sterile pin and a 10  $\mu\text{l}$  droplet of conidial suspension of isolate ME24 was placed on the wound. The first two leaf petioles of each plant were inoculated. After inoculation, plants were covered with tray covers to maintain high humidity for 48h (inoculated with ascospores) or 72 h (inoculated with conidia).

### *Disease assessment and quantification of *L. maculans* DNA in leaf petioles*

For leaf lamina inoculation with GFP-expressing *L. maculans* conidia, the growth of *L. maculans* along leaf petiole towards the stem was measured by GFP fluorescence. At 22 days post inoculation (dpi), the inoculated leaves were detached at the place where the petiole joins the stem. The leaves were viewed using a Leica MZ FLIII stereo-microscope. For observation of GFP fluorescence, the GFP2 filter from Leica Microsystems (Milton Keynes, UK) was used. The distance from the inoculation site on the leaf lamina to the leading GFP *L. maculans* hyphal tip in the leaf petiole was measured on each leaf.

For leaf inoculation with ascospores, the growth of *L. maculans* along the leaf petiole to the stem was measured by quantification of the pathogen DNA using quantitative PCR (qPCR). At 18 dpi, the inoculated leaves were detached at the place where the petiole joins the stem. The leaf petiole was cut and placed in a 15 ml tube to be freeze dried. Freeze-dried individual leaf petioles were ground into powder using a mortar and pestle. A 20 mg sub-sample (from each ground individual leaf petiole) was used for DNA extraction using a DNA extraction kit (DNAMITE Plant Kit, Microzone Ltd, West Sussex, UK). The amount of

*L. maculans* DNA in each leaf petiole was quantified using a SYBR green quantitative PCR with the primers LmacF and LmacR (Liu *et al.*, 2006). A Stratagene Mx3000P Real Time PCR machine was used for quantification of *L. maculans* DNA. Each reaction volume was 20 µl, including 0.6 µl of primers at a final concentration of 300 nM, 10 µl of SYBR Green JumpStart Tag ReadyMix (Sigma, UK), 0.08 µl of ROX internal reference dye and 50 ng DNA sample. The thermocycling profile consisted of an initial cycle of 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, 72 °C for 45 s and a read step at 83 °C for 15 s, then a dissociation stage (thermal profile: 95 °C 1 min, 60 °C 1 min, 95 °C 15 s) (Huang *et al.*, 2009)

For leaf petiole inoculation with conidia of isolate ME24, the growth of *L. maculans* in the stem was measured by assessing stem canker severity. At 15 dpi, each plant was assessed for symptoms at the inoculated site on the leaf petiole. At 47 dpi, stems of the inoculated plants were harvested. The stem of each plant was cut at the leaf scar of the inoculated leaf to assess the internal severity of stem canker on a 0-4 scale; where 0 = healthy; 1 = 1-25% stem cross-section necrotic; 2 = 26-50% stem cross-section necrotic; 3 = 51-90% stem cross-section necrotic; 4 = 91-100% stem cross-section necrotic. In addition, the time from inoculation to the appearance of first Phoma leaf spots (for leaf lamina inoculation) or the appearance of lesions on petiole and stem (petiole inoculation) were recorded for each experiment.

## Results

### *Growth of L. maculans in leaf petioles*

In controlled environment experiments, there was no difference between DH lines A30 and C119 in the visual appearance of Phoma leaf spot symptoms, for leaves inoculated with conidia or ascospores of *L. maculans*. There were differences between conidial inoculum and ascospore inoculum in the incubation period (time from inoculation to the appearance of symptoms), with the incubation period shorter for ascospores than for conidia. Phoma leaf spots were observed at 7 dpi for plants inoculated with ascospores but Phoma leaf spots were not observed on plants inoculated with conidia until 10 dpi. However, there was no difference between DH lines A30 and C119 in length of the incubation period.

On leaves of DH lines A30 and C119 inoculated with conidia of GFP-expressing *L. maculans*, growth of hyphae along the leaf petiole towards the stem was observed. There was significant difference ( $P < 0.01$ ) in distance grown by *L. maculans* along the leaf petioles towards the stems between DH lines A30 and C119 (Figure 2a). On leaves of DH lines A30 and C119 inoculated with ascospores of *L. maculans*, Phoma leaf spots were produced at all the inoculated sites and the growth of *L. maculans* along the leaf petiole towards the stem was observed and quantified using qPCR. There was a significant difference ( $P < 0.001$ ) between DH lines A30 and C119 in the amount of *L. maculans* DNA in the leaf petiole (Figure 2b). The amount of *L. maculans* DNA in leaf petioles of DH line A30 was greater than that in leaf petioles of C119.

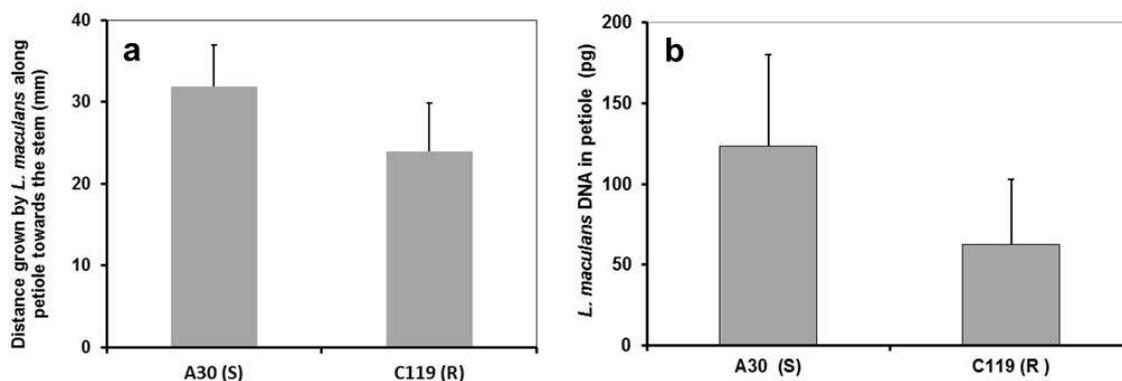


Figure 2. Growth of *Leptosphaeria maculans* in leaf petioles of doubled haploid lines A30 (susceptible) and C119 (good level of quantitative resistance). Leaves of DH lines A30 or C119 were inoculated with GFP-expressing conidia of *L. maculans* (a) or ascospores of *L. maculans* (b). Distance grown by *L. maculans* along leaf petiole towards the stem was measured by GFP fluorescence at 22 dpi (a) or by quantification of *L. maculans* DNA in leaf petioles using qPCR (data was square root transformed) (b). Bars are standard errors.

#### *Growth of L. maculans in stem tissues*

For leaf petioles inoculated with conidia of *L. maculans* isolate ME24, symptoms were observed at all the inoculated sites at 8 dpi. At 47 dpi, stem cankers developed on stems of both DH lines A30 and C119 at positions of leaf scars of the inoculated leaves. However, the internal severity of stem canker on DH line A30 was greater than on DH line C119 ( $P < 0.01$ ) (Figure 3).

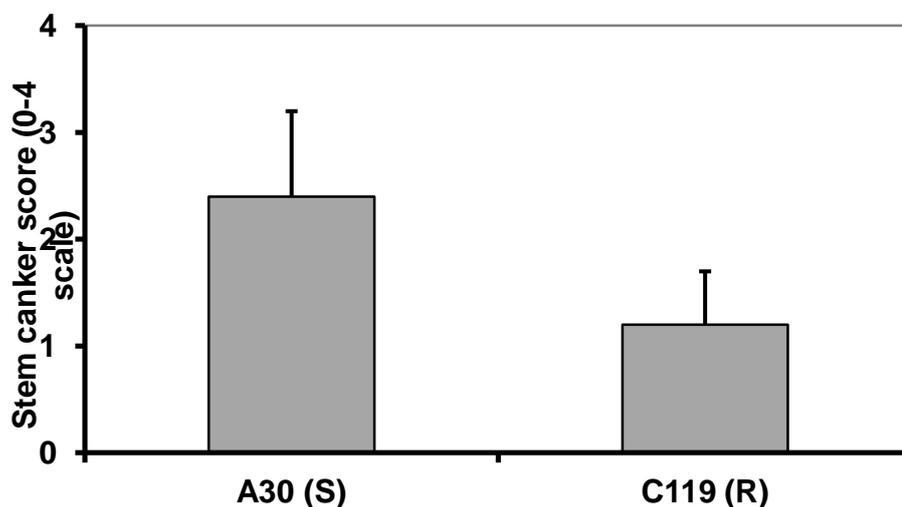


Figure 3. Internal stem canker severity score on doubled haploid lines A30 (susceptible) and C119 (good level of quantitative resistance) inoculated with conidia of *Leptosphaeria maculans*. Leaf petioles of DH lines A30 and C119 were inoculated with conidia of *L. maculans* isolate ME24. Stems of A30 and C119 were assessed for internal stem canker severity on a 0-4 scale at 47 days after inoculation. Bars are standard errors.

## Discussion

This work provides experimental evidence that components of quantitative resistance to *L. maculans* can be detected during the growth of the pathogen along the leaf petiole towards the stem or during colonisation stem tissues of *B. napus*. There were no differences in incubation period for Phoma leaf spot development between DH lines A30 and C119, suggesting that quantitative resistance to *L. maculans* does not operate during the initial short period of symptomless growth in the leaf lamina. This is consistent with previous work using cultivars Darmor (good level of quantitative resistance) and Eurol (susceptible) (Huang *et al.*, 2009). Investigation of symptomless growth of *L. maculans* in leaf petioles by quantification of the amount of *L. maculans* DNA in the petiole showed that the growth of *L. maculans* in leaf petioles of C119 was much less in leaf petioles of A30. This suggests that quantitative resistance to *L. maculans* may operate by slowing down the growth of *L. maculans* in the petiole towards the stem. However, this result was not observed when comparing Darmor and Eurol at the same growing stage (Huang *et al.*, 2009). The relationship between growth of *L. maculans* in the leaf petiole and quantitative resistance needs further investigation. Quantitative resistance to other plant pathogens often operates by reducing the growth of pathogen within the host. Quantitative resistance to *Mycosphaerella graminicola* (*Zymoseptoria tritici* blotch) in wheat, operates by decreasing the leaf area covered by lesions bearing pycnidia (Chartrain *et al.*, 2004), while quantitative resistance to *Magnaporthe grisea* (rice blast) operates by decreasing both number of leaf lesions and size of lesions (Talukder *et al.*, 2004). For both these pathogens, yield loss is associated with damage to the leaves, whereas for *L. maculans* it is associated with damage to the stems.

The results of leaf petiole inoculation experiments suggest that *B. napus* quantitative resistance to *L. maculans* also operates during colonisation of stem tissues by the pathogen. In controlled environments, less severe stem cankers developed on DH line C119 than on DH line A30. This suggests that quantitative resistance to *L. maculans* operates after the leaf spot stage by decreasing the subsequent growth of *L. maculans* in stem tissues. Previous work on using a GFP-expressing isolate to visualise symptomless growth of *L. maculans* within stem tissues showed that quantitative resistance operates by preventing or restricting the spread of *L. maculans* from the stem cortex to stem pith (Huang *et al.*, 2009).

Results of these experiments with different types of inoculum confirmed that ascospores of *L. maculans* are more infective than conidia, which is consistent with previous work (Huang *et al.*, 2006). Phoma leaf spots developed more rapidly on plants inoculated with ascospores than on plants inoculated with conidia. There is a long period of symptomless growth of *L. maculans* between the appearance of Phoma spots on leaf laminae and the appearance of Phoma cankers on stems (Hammond *et al.*, 1985; Fitt *et al.*, 2006; Huang *et al.*, 2009). This makes it difficult to produce stem canker symptoms in controlled environments by inoculation of leaf laminae. Results of these experiments with different inoculation methods suggest that the leaf petiole inoculation method is an efficient method for producing stem cankers in controlled environments. Recently, a cut petiole inoculation method was used to compare aggressiveness of different *L. maculans* isolates in terms of their ability to cause stem canker in controlled environments (Brun *et al.*, 2011). The development of methods for producing stem canker symptoms in controlled environments now makes it possible to screen for quantitative resistance to *L. maculans* at seedling stage. This method needs to be applied on a wider range of genotypes and on segregating progenies for quantitative resistance to assess correlation between controlled conditions and field evaluation as initiated by Delourme *et al.* (2011). This could help to accelerate the process of breeding cultivars with quantitative

resistance, which currently relies on assessment of stem canker at harvest (Pilet *et al.*, 1998; Delourme *et al.*, 2006; Fitt *et al.*, 2006).

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## Characterization of current populations of *Leptosphaeria* spp. from infected oilseed rape plants in Europe (autumn 2010)

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**Abstract:** Knowledge about the current populations of plant pathogens is important for combating the diseases they cause and decreasing yield of crop plants. The aim of this work was to characterize the population of *Leptosphaeria* spp. obtained from oilseed rape plants in Europe in the autumn of 2010.

The samples were collected from 20 experimental sites, including 9 fields in Poland, 5 in Hungary, 2 in Germany and France and 1 site in Denmark and the Czech Republic. The materials were collected from the cultivar PR46W10 (HiBred-Pioneer). The plots were not treated with fungicides. From each field, 55 leaves of oilseed rape with visible symptoms of phoma leaf spotting were collected at random. Leaf fragments colonized by the species of *Leptosphaeria* complex were surface disinfected with 70% ethanol and 1.5% sodium hypochlorite, rinsed in sterile distilled water and subcultured on PDA medium supplemented with 0.02% streptomycin sulphate. Single spore or hyphal tip isolates obtained from the studies were attributed to *L. maculans* or *L. biglobosa*, based on visual differences in culture morphology, confirmed with RAPD using primer OPJ-10. There were considerable differences in the proportion of both *Leptosphaeria* species obtained from the different sites, ranging from 14.3% of *L. maculans* (85.7% of *L. biglobosa*) in Hegyfalú, Vas region in Hungary to 97.1% of *L. maculans* (2.9% *L. biglobosa*) in Gut Barthof, Bayern region in Germany. The results of this study are contrary to current knowledge on *Leptosphaeria* species on oilseed rape in Europe. There were differences between the fields, but – in general – in central Europe (Poland, the Czech Republic and Hungary) the prevailing species was *L. maculans* (81%). In Germany and Denmark the isolates of *L. maculans* constituted a quarter of all *Leptosphaeria* cultures obtained from oilseed rape plants and in France the mean amount of *L. biglobosa* isolates was also high (25% to 33% depending on collection site). The variation between the composition of the populations of *Leptosphaeria* may significantly vary within a country, however, this investigation shows that the amount of *L. biglobosa* in west Europe may be higher than expected. One of the possible reasons may be connected with the higher persistence of *L. biglobosa* in oilseed rape plants and difficulties to eradicate this species with lower doses of fungicides. Once developed on stems, *L. biglobosa* forms numerous pycnidia and then pseudothecia and produces big numbers of ascospores (primary inoculum).

In addition, the isolates of *L. maculans* obtained from Poland were ascribed to the opposite mating types and (a)virulence alleles. The population still favours *Mat 1.2* (66%) as compared to *Mat 1.1*. The *avrLm1* allele was still dominating (90%) comparing to *AvrLm1*. In spite of no cultivars with *Rlm6* resistance gene, we have found 5 isolates with *avrLm6* allele. Strangely, these isolates were not localized in west Poland, where they could be transmitted with west winds, but at two locations in the central part of the country. The detection of *AvrLm6* was done using PCR. The cotyledon test checking the virulence of the isolate to *Rlm6* resistance gene present in Darmor MX and Eurol MX cultivars is currently under way.

**Key words:** *Brassica napus*; Phoma stem canker; population; species ratio; mating types; avirulence genes

## **Comparative studies on growth and fungicide sensitivities of *Leptosphaeria maculans* and *Leptosphaeria biglobosa* isolates**

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**Abstract:** Oilseed rape is affected by a *Phoma* species complex causing blackleg or stem canker. The two teleomorphs of *Phoma lingam*, *Leptosphaeria maculans* and *L. biglobosa* are associated with this disease. These species are believed to be of different economic importance. Stem base girdling and severe stem cankers are assigned to *L. maculans*. However, *L. biglobosa* can also be isolated from stem bases, but it is more frequently found at higher stem areas. These observations suggest different biological properties of the two species. In this context we compared temperature demands of three isolates of each species between 5-32 °C, recording radial mycelial growth on malt extract agar. Our results suggest a broader temperature range and a more pronounced growth of *L. biglobosa* at lower temperatures. Numerous studies demonstrated higher radial mycelial growth rates of *L. biglobosa* compared to *L. maculans* on different media. This observation may not be based on differences of biomass production. Huang *et al.* (2001) studied ascospore germination of these two species and observed different mycelial growth patterns. Whereas, *L. maculans* displayed pronounced branching, *L. biglobosa* shows only faint branching of hyphae, which may pretend higher biomass production by more straight hyphae. To assess the capacities of biomass production of both species, we recorded dry matter production of three isolates each after different growth periods in liquid culture. Results show that both species were not significantly different.

Finally, the isolates of both species were checked for their sensitivity against 16 fungicides *in vitro*. For this purpose fungicides were implemented into Czapek Dox media at 1, 1:5 and 1:5 field application rates. Radial mycelial growth was recorded at regular intervals after incubation at 20°C in the dark. Active compounds of the sterol biosynthesis inhibitors (FRAC groups G1 and G2) Metconazol, Difenoconazol, Tebuconazol, Prothioconazol, Fenpropimorph, Spiroxamine, Fenpropidin) were highly effective against all studied isolates. This was also true for carbendazim (B1), bixafen (C2) and iprodion (E3). Insensitivities were observed for cyflufenamid (6), metrafenone (U8) and proquinazid (E6). Efficacy of fungicides of the group of strobilurins (C3) varied significantly. Whereas pyraclostrobin still shows efficacy, azoxystrobin was only effective against 30% of the isolates tested. Boscalid (C2) also showed reduced efficacy at field application rates in contrast to iprodion (E3), which was effective.

**Key words:** oilseed rape; *Phoma* stem canker; fungicide efficacy; radial mycelia growth

### **Reference**

Huang, Y. J., C. Toscano-Underwood, B. D. L. Fitt, A. D. Todd, B. Koopmann, M. H Balesdent 2001: Effects of temperature on germination and hyphal growth from ascospores of A-group and B-group *Leptosphaeria maculans* (*Phoma* stem canker of oilseed rape). *Annals of Applied Biology* 139(2): 193-207.

## Molecular detection of *Leptosphaeria maculans* and *L. biglobosa* versus BBCH stages of oilseed rape plant development – the impact on protection with fungicides

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**Abstract:** Winter oilseed rape (*Brassica napus* ssp. *oleifera*) became one of the most profitable crops grown in Europe. Its plants stay in the field for 11 months, and during this long period they can be attacked by numerous diseases, including blackleg or stem canker. The disease is caused by two highly related pathogenic fungal species: *Leptosphaeria maculans* [Desm.] Ces. et de Not. and *L. biglobosa* sp. nov. (Shoemaker & Brun). The fungus is transmitted by airborne ascospores, that land on leaves and cause plant infection. In Europe, primary infection takes place in the autumn. The beginning of spore release and the time of the maximum concentration of spores in air samples greatly depend on weather conditions. The collection of ascospores from air samples was done in Wielkopolska region (Poznan, Great Poland) over five consecutive seasons (2004-2008) from 1st September to 31st November. The weather data were collected from the optimal sowing time (20 August) till the end of spore sampling session. The detection of spores belonging to each species of the *Leptosphaeria* complex was done based on quantitative PCR method using species-specific dual-labelled fluorescent probes designed based on  $\beta$ -*tubulin* genes. In three consecutive autumn seasons (2004-2006) the summary number of spores of *L. maculans* was higher than this of *L. biglobosa*, in autumn 2007 it was twice bigger for *L. biglobosa*, and in autumn 2008 it was equal for both species. The beginning of spore detection of the two species was earlier for *L. biglobosa* two times (2004, 2005), in the other two seasons (2007, 2008) it was identical for both species and in autumn 2006 it was earlier for *L. biglobosa*. These differences in time ranged from 8 to 11 days. The detection of the maximum number of spores of these two species was earlier for *L. maculans* in three seasons (2004, 2006, 2008) and slightly earlier (11 days) for *L. biglobosa* in autumn 2005. These differences in time ranged from 3 to 18 days. In 2005 there were two identical peaks of the maximum concentration of *L. biglobosa* in this season. In autumn 2007 the mass ascospore release was identical for both species. The number of days with spores of *L. maculans*, present in air samples ranged from 9 to 48, and for *L. biglobosa* it ranged from 9 to 39, which indicates that the differences between seasons were large (9.9% to 49.5%). Ascospore showers of *L. maculans* and *L. biglobosa* differed greatly from each other and coincided with different stages of development of oilseed rape plants, measured using the BBCH scale. The growth stage of oilseed rape plants and the efficiency of the fungicide to both species have strong impact on the protection of oilseed rape, because both species show different reactions to the same dose of the fungicides and their active compounds, with *L. biglobosa* more persistent and difficult to eradicate.

**Key words:** *Brassica napus*; Phoma stem canker; spore dispersal; quantitative PCR

## **Studies on the optimal time of fungicide application against Phoma leaf spotting and stem canker in Poland**

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**Abstract:** Among the diseases that affect oilseed rape, one of the most damaging is Phoma leaf spot and stem canker. It is caused by two species of fungi – *Leptosphaeria maculans* and *L. biglobosa*, respectively. The main source of plant infection are ascospores originating from pseudothecia – fruiting bodies of the perfect stage, produced on oilseed rape stubble from the previous vegetative season. These wind dispersed spores constitute the inoculum for the spread of the disease from season to season and to new fields in the same season. Monitoring of spore dispersal is of great importance for disease risk assessment. In Poland this monitoring has been done within the framework of System for Forecasting Disease Epidemics ([www.spec.edu.pl](http://www.spec.edu.pl)) using volumetric spore samplers.

The aim of this research was to determine the effect of the autumn and early spring spraying time on the incidence and severity of stem canker in relation to presence of spores in the air. The experiments were done in three seasons: 2008/2009-2010/2011. The fields with winter oilseed rape cultivar PR46W10 (Pioneer Hi-Bred) were placed in 5 sites located in different regions of Poland. Fungicide treatments were done at weekly intervals from late September to mid-November, using Capitan 250 EW, containing 250 g of flusilazole per 1 l of the fungicide.

The results clearly show that time of fungicide application had a strong, statistically significant influence on the effectiveness of chemical protection of oilseed rape. The percentage of healthy plants was significantly different between assessment dates, years and locations. The lowest percentage of healthy plants was always observed in variants with no fungicide treatments. Application of fungicide was the most efficient when it was performed a few days following the ascospore release. It caused not only reduced disease incidence but also the highest increase of the yield.

In conclusion, information about timing and intensity of ascospore release given by SPEC may greatly help in controlling stem canker epidemics and allow better predictions of the disease risk.

**Key words:** oilseed rape; *Leptosphaeria maculans*; *Leptosphaeria biglobosa*; spore dispersal

## Effect of combined inoculation of *Phoma lingam* pathogenicity groups on disease expression of cotyledons of a *Brassica napus* Rlm7 cultivar

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**Abstract:** Pathogen resistance is the most important measure for integrated control of fungal diseases. Both qualitative (monogenic) and quantitative (polygenic) resistance genes contribute to blackleg resistance of oilseed rape (OSR). Several monogenic resistance genes are known and widely used in commercial cultivars. Recently, a few cultivars were introduced into the market possessing the new monogenic resistance gene Rlm7, which has been shown to be highly effective. However, monogenic resistance is vulnerable to being overcome by the development of virulent fungal populations. This process may rely on the selection of virulent isolates already present in the population at low frequencies, but also on avirulence genes which may alter and turn into virulence alleles or are deleted via mutation. We isolated *Leptosphaeria maculans* avr7-strains virulent on Rlm7 genotypes from experimental plots in Göttingen. In this study we used an isolate to investigate the performance of Rlm7 if triggered with isolate combinations of different pathogenicity groups. It has been shown in OSR and other hosts, that pre- and/or co-inoculations with less virulent isolates may induce resistance to highly virulent isolates. We studied the presence of this effect in the Rlm7 cv. Caiman using well characterized isolates of both *L. maculans* and *L. biglobosa*.

Pre-inoculations were performed with an Avr7 *L. maculans* or an *L. biglobosa* isolate both avirulent on cv. Caiman. They were followed by inoculations with a virulent *L. maculans* isolate (avr7) 0, 1, 2 and 4 days after pre-inoculation. A mock pre-inoculation control was also realized. Different spatial inoculation systems were used to check for local and systemic effects of induced resistance. Local effects were studied by inoculating both isolates at the same place or each on a separate half of a cotyledon. Systemic effects were studied by inoculating avirulent and virulent isolates on different cotyledons of a single plant. Both lesion area and qualitative assessments considering sporulation of the pathogen were conducted. For this purpose we used the IMAScore rating scale (Volke, 1999).

Results clearly demonstrate efficacy of Rlm7 against both studied *L. maculans* Avr7 and *L. biglobosa* isolates. Lesion sizes were about 50 times smaller compared to *L. maculans* avr7-controls. Also no sporulation was observed within these lesions after 18 days post inoculation. Results of combined inoculations showed clear effects only if isolates were inoculated in close proximity. This was true for both pre-inoculation systems using either avirulent *L. maculans* or *L. biglobosa*. This effect was even more pronounced and statistically significant compared to the controls, if there was a time gap of 4 days between pre-inoculation and inoculation. This variant also showed no sporulation of the pathogens. Lower effects were recorded when inoculations were spatially separated. Slight effects were only visible using Avr7 *L. maculans* for pre-inoculations. However, they were not statistically different from controls. No effects, not even a trend, were observed using *L. biglobosa* for spatially separated pre-inoculations.

These results demonstrate that Rlm7 may be triggered by avirulent *L. maculans* and *L. biglobosa* locally. Probably, a systemic effect of a pre-inoculation with avirulent *L. maculans* and *L. biglobosa* becomes significant in systems using extended time gaps between pre-inoculation and inoculation.

**Key words:** oilseed rape; Rlm7; induced resistance; *Leptosphaeria maculans*; *Leptosphaeria biglobosa*

## Reference

Volke, B. 1999: *Leptosphaeria maculans*, der Erreger der Wurzelhals- und Stengelfäule an Raps: Verbreitung verschiedener Pathogenitätsgruppen in Europa, Quantifizierung des Befalls und Schadwirkung im Freiland. PhD thesis, University of Göttingen, Germany.

## Characteristics of isolates of *Sclerotinia sclerotiorum* and *Leptosphaeria maculans*/*L. biglobosa* originating from the Czech Republic

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**Abstract:** In 2011, samples of the fungi *Leptosphaeria* spp. and *Sclerotinia sclerotiorum* were isolated and grown in pure cultures on potato dextrose agar (PDA). The isolates were taken from different plant tissues of winter oilseed rape (*Brassica napus*). The *Leptosphaeria* spp. isolates were taken from leaves whilst sclerotia of *S. sclerotiorum* were found in the stems at harvest. Samples originated from different sites. The type of *Leptosphaeria* spp. was detected using PCR and isolates were confirmed as either *L. maculans* or *L. biglobosa*. In culture, differences were observed for different *Sclerotinia* isolates with respect to mycelial growth rate and the size of sclerotia that were formed. Isolates of the three pathogens will be used in field tests to monitor levels of resistance in different cultivars of oilseed rape and to evaluate the efficacy of fungicides.

**Key words:** oilseed rape; PCR differentiation; culture characteristics



## ***Sclerotinia* stem rot**



## ***Sclerotinia sclerotiorum* – the important disease of oilseed rape on selected sites in the Czech Republic**

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**Abstract:** *Sclerotinia* stem rot is a serious global problem on oilseed rape. The disease is caused by the polyphagous pathogen *Sclerotinia sclerotiorum* which also attacks sunflower, mustard, peas, beans and various other vegetable crops (tomatoes, cucumbers, lettuce etc.). Since 2007, serious oilseed rape diseases (*Sclerotinia* stem rot, blackleg etc.) have been monitored at two locations in the Czech Republic. Research institutions in Šumperk and Opava are engaged in testing for the occurrence of *Sclerotinia* ascospores on oilseed rape petal leaves. Tests are done according to the methodology used at the University of Saskatchewan (Petal Test Manual for *Sclerotinia* in Canola; 1991). The testing of petals for the presence of ascospores is done during the flowering period and the data are used to predict the possible level of infection in selected regions of the Czech Republic. We advise growers when they should treat oilseed rape crops with the aim of decreasing plant damage and yield loss. Since 2004 we have also tested the resistance of selected varieties of winter oilseed rape to *Sclerotinia* stem rot. This work has produced a large dataset that includes information on resistance, overwintering and frost-proof ability, yield and thousand grain weight (TGW) from a number of experiments across the Czech Republic. This project is funded by Czech Ministry of Agriculture (grant 111A075).

**Key words:** *Sclerotinia sclerotiorum*, oilseed rape, testing, ascospores

## **Interactions between canopy structure of winter oilseed rape and *Sclerotinia sclerotiorum* disease development**

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**Abstract:** *Sclerotinia* stem rot caused by the fungal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most damaging diseases of oilseed rape (*Brassica napus*) with harmful attacks 2 in 3 every 10 years. Currently, this fungal disease is the major cause of fungicide applications on the crop. In order to find alternative approaches to pesticides as expected by the French Ecophyto 2018 plan, we are looking for agronomic practices able to limit attacks of *Sclerotinia* on oilseed rape.

The following hypothesis was tested: an “open” canopy in oilseed rape could limit the development of disease by reducing the contact between infested petals (pathogen) and leaves (host), and by creating a canopy microclimate unfavorable for disease development. Contrasted canopies have been generated using 3 factors: plant density, variety and nitrogen date of application during two agronomic seasons in several locations each year.

During the oilseed rape development, some observations were made to control plants' growth, to estimate differences between canopies (ramifications, LAI), to estimate disease development risk (infested petals' rate, petals fallen in the canopy, measure of climate within the canopy). Disease incidence and severity under natural contamination have been checked when it occurred.

Variance analyses bring to light morphologies of contrasted canopy according to the tested modalities, more or less favorable of petals' adhesion to leaves. Lower densities develop canopies with more ramifications: revealing the plasticity of the plant able to compensate for low densities. These differences of canopy architecture are to be connected with the fungus development.

**Key words:** *Brassica napus*; stem mold; plant density; nitrogen supply

## Improvement of stem mold resistance of oilseed rape

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**Abstract:** The German set of winter oilseed rape varieties does not contain any recognised resistance against the pathogen *Sclerotinia sclerotiorum*. Because of this, screening methods were developed to characterise a set of varieties provided by different breeding companies. These methods, including field experiments, a greenhouse screening study and a laboratory assay, were presented and a comparison of results given. Additionally the greenhouse screening and the laboratory assay were used to characterise wild *Brassica* species. By these means, possible sources of resistance should be identified.

**Key words:** *Brassica napus*, *Sclerotinia sclerotiorum*, resistance

### Introduction

Against *Sclerotinia sclerotiorum* (Lib. de Bary) no real resistance genes are available in German oilseed rape (OSR) varieties. This is due to two factors. First there are no strong sources for resistance against this pathogen available in the germplasm. It is well known that some sources of resistance are available in Chinese cultivars but this material has not been incorporated into elite German breeding lines so far. The second problem is the lack of a screening method for the evaluation of resistance against this pathogen. A screening method suitable for the breeding of new OSR cultivars should be reliable and fast enough to allow the rating of a high number of genotypes in a short period of time. Thus the aim of this study was to develop screening methods which fit these criteria by using a set of genotypes which was provided by participating breeding companies. Suitable methods for the rating of resistance in the field, under greenhouse conditions and in the laboratory were developed. The laboratory assay relies on the reaction of leaf material on oxalic acid, the main pathogenicity factor of *S. sclerotiorum*. These methods should be used for the identification of possible sources of resistance in the breeding material or in other gene pools.

### Material and methods

#### *Field experiments*

Field experiments were conducted over two years at three locations. At Göttingen, four replications were assigned in a randomized design while at the two other locations, near Peine and on the isle of Poel, just three replications were used in a randomized block design. In each experiment 28 genotypes were used. These were provided by participating breeding companies. These genotypes should display the biggest differentiation in the trait *Sclerotinia* resistance which is available to the participating breeding companies. Routine cultural practices were used at each site. Plots were split into two. One part was treated with a fungicide at the time point of

flowering. In the other part of the plot no fungicide was applied. Approximately 4 g of sclerotia per m<sup>2</sup> were added to plots in autumn to achieve a high infestation level. The rating of disease incidence was done by counting the infected and non-infected plants on 4 m<sup>2</sup> per plot at the time of pod ripening and calculating the percentage of diseased plants.

### ***Greenhouse screening***

After vernalisation, plants were cultivated in a greenhouse at 25 ± 10 °C until they reached BBCH Stage 65 (Meier, 2001). During cultivation the plants were watered and fertilised as required. When plants reached BBCH 65, they were inoculated by placing an agar plug with fresh mycelium into a leaf axil. The plugs were fixed with parafilm, to prevent them from drying. The agar plugs (Ø 7 mm) were cut from the edge of a three days old culture of *S. sclerotiorum* growing on PDA. These plates were cultured at 20 °C. Three days after inoculation the plugs and the parafilm were removed and the length of the developing lesion was measured. The same measuring was done daily until 9 dpi. These lesion lengths were used to calculate the AUDPC according to Kaiser, 2007. In some cases no lesion could be observed after three days, so the plants were inoculated again on a different location until a lesion was visible. The number of inoculations which was needed to achieve a visible lesion was recorded and used to calculate the inoculation efficiency. To calculate this the following formula was used:

$$\text{Inoculation efficiency [\%]} = \frac{\# \text{ Lesions}}{\# \text{ Inoculations}} * 100$$

### ***Oxalic acid treatment***

Plants for the oxalic acid assay were cultured at 20 °C without fertilization until the fifth true leaf was fully developed. During the cultivation, plants were watered as required. On the day before the oxalic acid treatment all plants were watered, independently on the moisture of the soil, to realize a homogenous water status of all plants. For the oxalic acid treatment leaf discs (Ø 1.3 cm) were cut from the fifth true leaf. These discs were incubated in 2 ml of 2 mM oxalic acid for two hours. Additionally discs were incubated in a control solution of distilled water. After incubation, the acid and the water was carefully removed without wounding the leaf discs. After three washing procedures with distilled water, 2 ml of distilled water were added to each leaf disc. In this water the conductivity was measured and the discs were incubated at 20 °C for 24 hours. Afterwards the conductivity of the water was measured again and any change in conductivity was calculated according to the following formula:

$$\Delta S = S_{2\text{mM};24\text{h}} - \bar{x}S_{2\text{mM};0\text{h}} - (\bar{x}S_{0\text{mM};24\text{h}} - \bar{x}S_{0\text{mM};0\text{h}})$$

where  $\Delta S$  is the change of conductivity corrected by the change of conductivity in the water treatment,  $S_{2\text{mM};24\text{h}}$  is the conductivity in the 2 mM oxalic acid treatment after 24 hours of incubation,  $\bar{x}S_{2\text{mM};0\text{h}}$  is the mean conductivity of the 2 mM oxalic acid treatment before incubation,  $\bar{x}S_{0\text{mM};24\text{h}}$  is the mean conductivity of the water treatment after 24 hours of incubation and  $\bar{x}S_{0\text{mM};0\text{h}}$  is the mean conductivity of the water treatment before incubation.

Additionally the number of stomata per mm<sup>2</sup> was determined in the experiment in which the wild *Brassica* species were used. The change of conductivity was divided by the number of stomata of the single cultivar and thus used as an additional correction factor.

## Results and discussion

Figure 1 shows the percentage of infected plants at Göttingen in the year 2008/09 in the two treatments. A differentiation between the cultivars is visible but there are no extremely resistant or susceptible cultivars which show a big diversity in the reaction against the pathogen. One should also keep in mind, that the infestation level overall was not very high. A similar result was observed in the year 2009/10 and at the two other locations.

This is consistent with data which are available for commercially available OSR cultivars in Germany. Both, the Beschreibende Sortenliste (Anonymus, 2009) as well as the UFOP Sortenversuche (Union zur Förderung von Oel- und Proteinpflanzen e.V. (UFOP), 2010), describe just small differences in tested genotypes. Thus it is not surprising that no big differences can be found between the genotypes which were used in the field experiments.

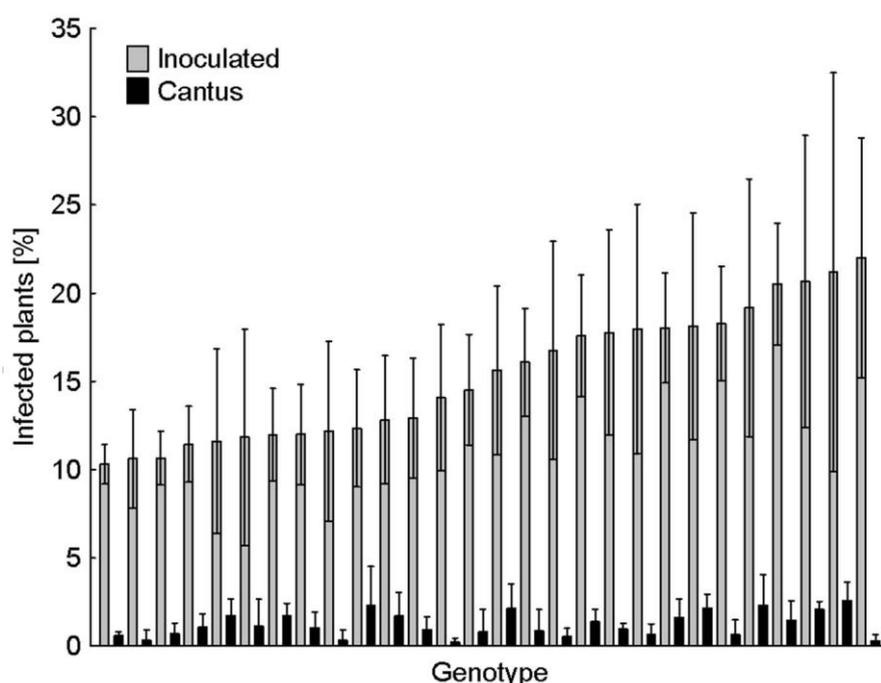


Figure 1. Infected plants [%] in the field experiment 2008/09 at Göttingen in the two treatments Cantus and Inoculated for the used cultivars with standard deviation.

To get more results for the resistance reaction of the genotypes, a greenhouse screening method was developed. Here the speed of lesion development was measured and used to calculate the AUDPC values for the genotypes which were also used in the field experiment. As can be seen in Figure 2, differentiation between the genotypes was improved when using the AUDPC values compared with the infestation level in the field experiment. Nevertheless no very resistant or susceptible cultivar could be identified. The differentiation which was achieved in this experiment shows that it is possible to screen cultivars under greenhouse conditions. The biggest advantage of this method is that it is not dependent on climatic conditions which might have an influence on the infestation level in the field. Moreover, some disease escape mechanisms like e.g. the time point of flowering are not considered in this greenhouse screening. Thus a more precise evaluation of the resistance is possible.

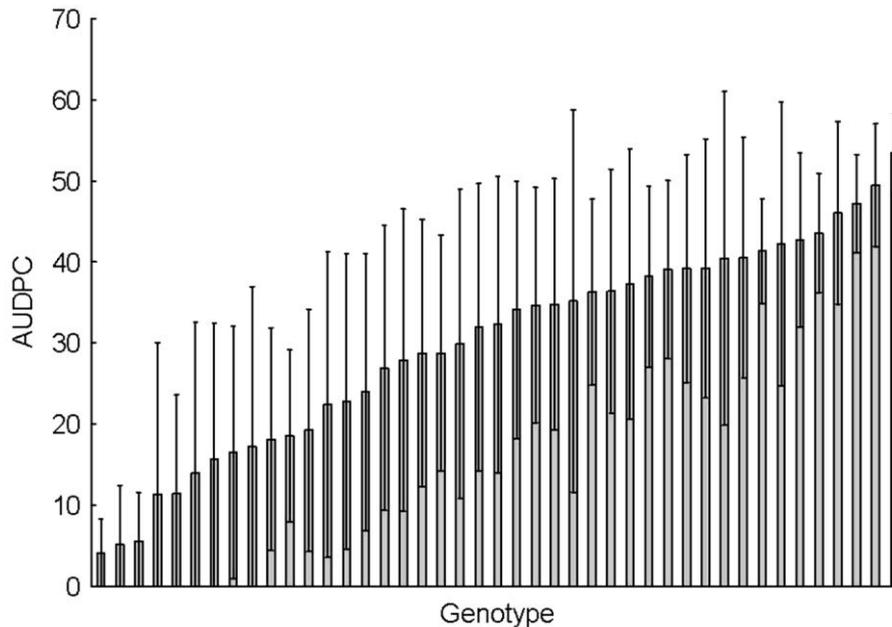


Figure 2. AUDPC values calculated from the lesion length in the greenhouse after artificial inoculation for oilseed rape genotypes with standard deviation.

The biggest disadvantage of field experiments and the greenhouse screening in this context was the time which was needed to evaluate winter OSR genotypes. Because of this, a method was developed which should be able to give an indirect prediction of resistance in a very short period of time. This method, the oxalic acid assay, indicated the reaction of leaf material against the most important pathogenicity factor of the fungus. Oxalic acid damages the cell membranes of the treated leaf discs. Ions leak out from the damaged cells and influence the conductivity of the water in which the leaf discs were subsequently incubated. Assuming different reactions against oxalic acid also different levels of cell damage could be observed in different cultivars which would lead to differential changes of conductivity of the solutions. This was measured and the data are shown in Figure 3. Again, a differentiation between the oilseed rape cultivars is visible. A similar assay was conducted by Walz *et al.* (2008). Walz was able to show differences in the response of susceptible and resistant cultivars similar to the results shown here. Tu (1989) also used oxalic acid in a comparable concentration to show different levels of damage in the plasma membrane of different genotypes. Similarly, Chipps *et al.* (2005) were able to show differences in wilting symptoms after an incubation of bean leaflets in oxalic acid. The rating of resistance was similar to the wilting symptoms observed in this study.

Three methods have been developed and described which are suitable to screen for resistance of winter oilseed rape cultivars against *S. sclerotiorum* in different environments. The next step was to combine the results derived by the different screening methods but no significant correlation could be found. There might be several reasons for this outcome. One should keep in mind, that different parameters have been evaluated in the screenings. While the infestation level was measured in the field experiment, the speed of lesion growth was analysed in the greenhouse screening. The speed of growth was probably more correlated to the disease severity than to the incidence. This could explain the missing correlation between the results. In the oxalic acid assay, the reaction of plant material against just one pathogenicity factor of the fungus was measured. Other pathogenicity factors may also play an important role in the resistance of cultivars.

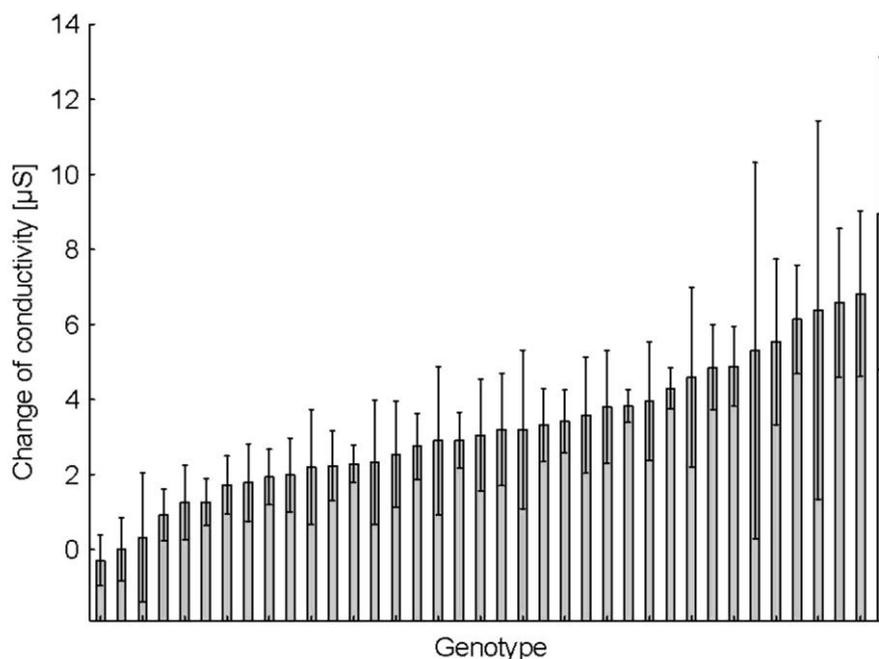


Figure 3. Change of conductivity [ $\mu\text{S}$ ] corrected by the conductivity at the beginning of the experiment and the conductivity in the water treatment with standard deviation.

Another possible explanation for the lack of a correlation would be an insufficient differentiation between the cultivars tested. When no differences in the reaction against the pathogen are available in the breeding material it is impossible to establish a significant correlation between the results. To test this, we tried to find material with a higher level of differentiation. This material was found in a set of wild *Brassica* species and resynthesized OSR. Because most of the wild species originated in the Mediterranean area, it was unlikely that they would survive the winter under German weather conditions. As such, the genotypes were not evaluated under field conditions and only a greenhouse experiment and the oxalic acid assay were done with these genotypes. During the greenhouse experiment again the AUDPC values were evaluated and are given in Figure 4. There was a striking difference between the original material and this new material which exhibited very low AUDPC values. The group comprises mainly wild species, while the resynthesized OSR cultivars exhibited higher AUDPC values.

In the greenhouse screening experiment, some genotypes showed no lesions after inoculation. These genotypes were inoculated again and the number of inoculations which was needed to create a lesion was used to calculate the inoculation efficiency (Figure 5). Big differences can be found between the genotypes tested. In accordance to the AUDPC values in the inoculation efficiency also a grouping of the genotypes can be found. While the resynthesized OSR cultivars exhibited comparatively low inoculation efficiencies, the wild species showed higher values which leads to the conclusion that there has to be a difference in resistance mechanisms.

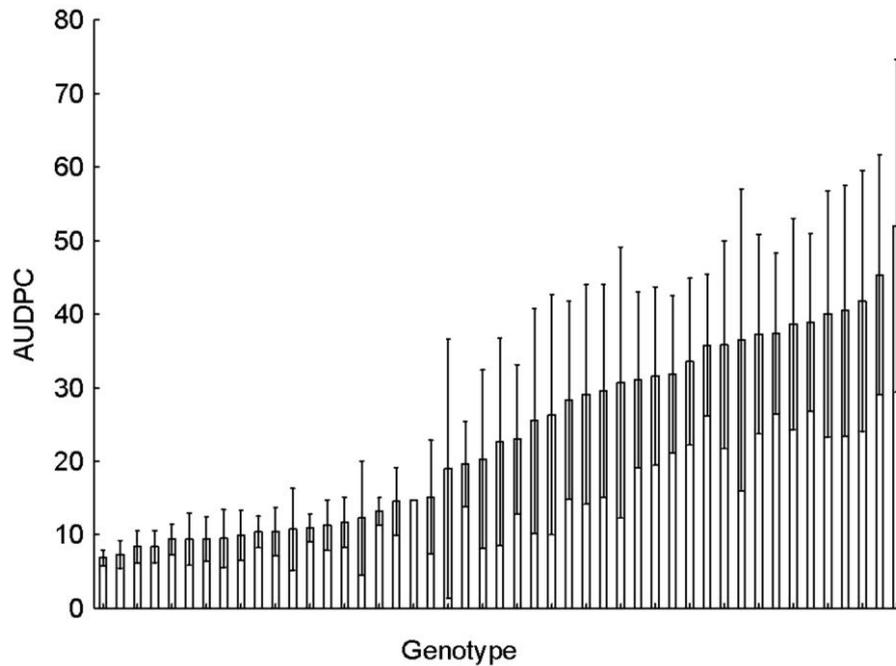


Figure 4. AUDPC values, calculated from the lesion length in the greenhouse after artificial inoculation for the different wild *Brassica* species and resynthesized OSR with standard deviation.

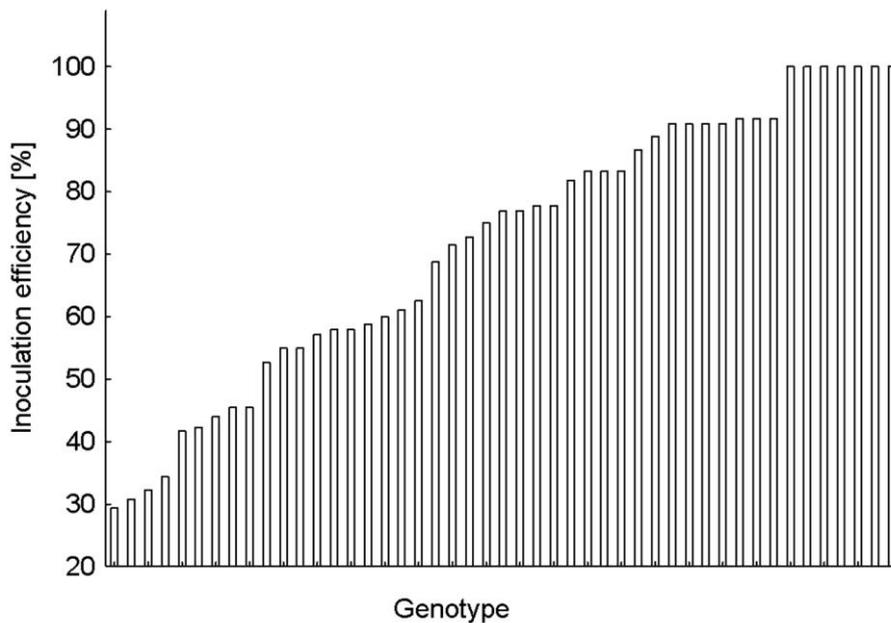


Figure 5. Inoculation efficiency in the greenhouse after artificial inoculation of different wild *Brassica* species and resynthesized OSR with *Sclerotinia sclerotiorum*.

Different inoculation efficiencies would result in different infestation levels in the field. Kim and Cho (2003) were able to detect such differences between cultured *Brassica* species which is in accordance to the results described here. Wild species were also used by Mei *et al.* (2010). They were able to show a big diversity in lesion development between the different wild species tested. This is in agreement with the above described results.

A significant correlation was found between inoculation efficiency and AUDPC values in the set of wild species and resynthesized OSR genotypes (Figure 6). On closer examination, it was obvious that different groups can be found in this set of material. The first group exhibited low AUDPC values but high inoculation efficiencies. The second group showed higher AUDPC values and lower inoculation efficiencies. The last group had high values for both parameters and thus showed the highest susceptibility. When looking at the genotypes in the described groups it is striking that the first group comprises the wild *Brassica* species while *Brassica oleracea* and resynthesized OSR genotypes are found in the second group. However, the third group also was comprised of *B. oleracea* and resynthesized OSR genotypes but all contained *B. oleracea* as a parent. This results again points to different resistance mechanisms in the genotypes tested. While the wild species seem to have a quantitative physiological resistance, the resynthesized OSR genotypes seem to possess a mechanism which reduces the efficiency with which the fungus penetrates. Both mechanisms could be used in further breeding programs, but to use them the right screening method would need to be chosen. While the field experiment described above would just be suitable for the rating of the infestation level, the greenhouse screening could be used to rate both mechanisms of resistance.

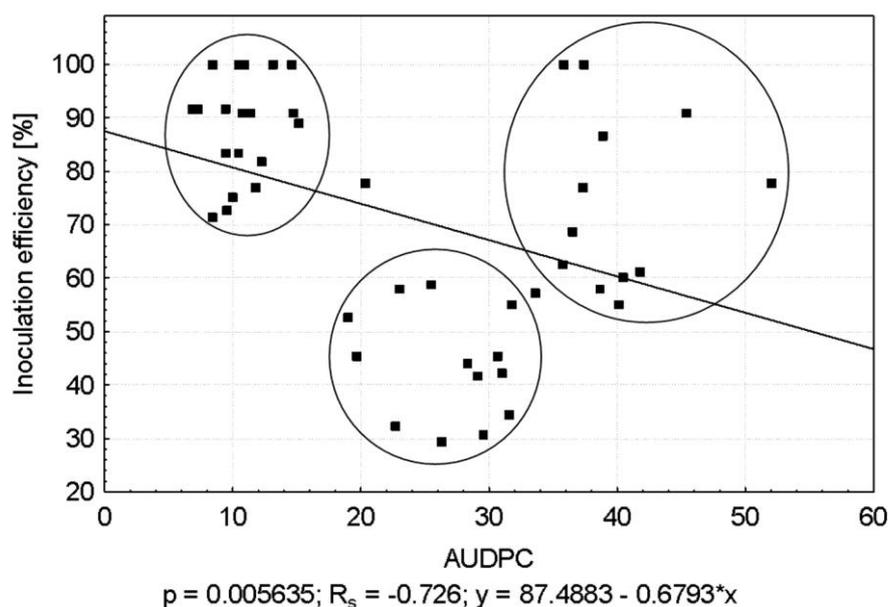


Figure 6. Correlation between Inoculation efficiency and AUDPC values in the greenhouse for tested wild *Brassica* species and resynthesized OSR. Ellipses indicate distinct groups.

A similar negative correlation between lesion development and infestation level has been described by Auclair *et al.* (2004), Chun *et al.* (1987) and Wegulo *et al.* (1998) in soybean. This implies that the observed mechanism can also be found in other plant species. This assumption is underlined by Vuong *et al.* (2004) who were able to show a similar relationship not only in soybeans, but also in sunflower.

To check whether these results could be reproduced and to evaluate the significance of the oxalic acid assay, some of the wild species and resynthesized OSR cultivars were also tested using that assay. The results, which are given in Figure 7 again indicate a good differentiation between the genotypes tested. Moreover the grouping which was described in the greenhouse screening can here also be found. The wild species exhibit a lower change of conductivity

compared to the resynthesized OSR genotypes tested. This is consistent with the results from the greenhouse screening, where the wild species exhibited a lower AUDPC value. A spearman rank correlation was done which indicated a significant correlation between the results ( $R_s$ : 0.607). This demonstrates the reproducibility of the results described and the different screening methods.

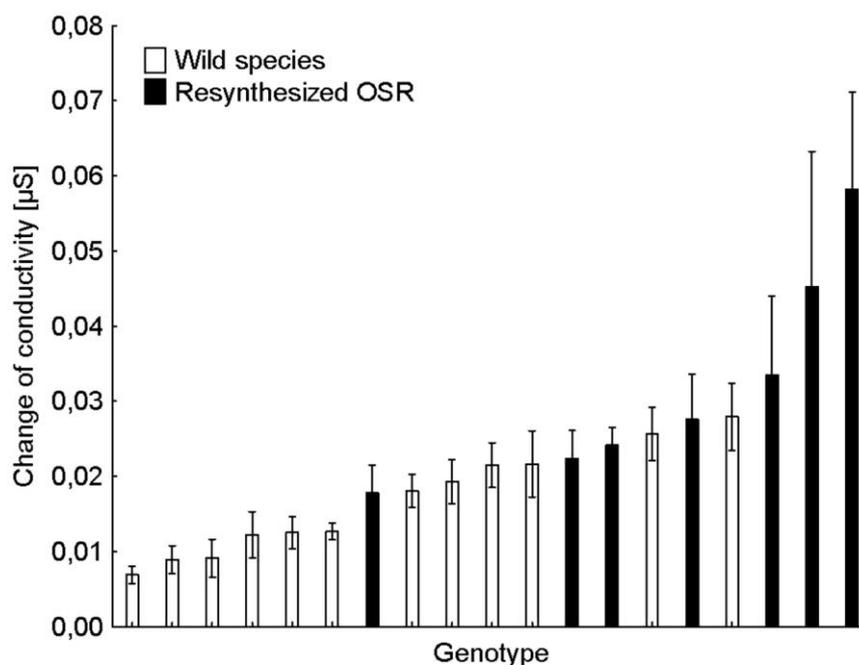


Figure 7. Change of conductivity [µS] corrected by the conductivity at the beginning of the experiment, the conductivity in the water treatment and the number of stomata for the used wild rassicca species and resynthesized OSR with standard deviation.

In conclusion, three different screening methods have been developed which are suitable for the screening of OSR cultivars for resistance to *S. sclerotiorum*. Moreover, wild *Brassica* species have been identified which show a high differentiation in the trait and could be used for further breeding programs.

## Acknowledgements

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## Use of geographic information systems for the DSS SkleroPro - simulation of *Sclerotinia* stem rot

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**Abstract:** In 2010, a new way of presenting results of Decision Support Systems (DSS) for plant pests has been implemented in the Web Information System for Integrated Plant Production (ISIP) [www.isip.de](http://www.isip.de). By means of Geographic Information Systems (GIS), meteorological data of temperature, relative humidity and global radiation were interpolated. Additionally precipitation data from radar measurements in a high spatial resolution were used as input parameter for the simulation models. This data lead to risk maps which help to identify hot spots of disease infection or pressure and simplifies the interpretation of the models results. Furthermore the user does not have to choose a specific meteorological station, but gets a field-specific calculation for his plant production site with spatial resolution of 1 km<sup>2</sup>. The system is supplemented by a spatial three-day weather forecast offered by the German Meteorological Service. Since 2010 risk maps are implemented for the DSS SkleroPro – simulation of *Sclerotinia* stem rot.

**Key words:** Interpolation, GIS, temperature, relative humidity, risk maps, DSS, integrated control

### Introduction

Weather based simulation models are important modules in decision support systems (DSS) to control plant diseases. Several simulation models have been developed and introduced into agricultural practice in Germany (Racca *et al.*, 2009; Jörg & Bartels, 2008). To run this simulation models met. data e.g. temperature, relative humidity and precipitation recorded automatically from met. stations are necessary. In some agricultural areas, however, the distance between the met. stations and the field is more than 60 km. Those met. data are not representative for the field and do not lead to satisfying model results (Zeuner, 2007; Racca *et al.*, 2011).

A technical method helpful to solve this problem is the use of Geographic Information Systems (GIS). A field specific classification of met. data was calculated with complex statistical interpolation methods for the parameters temperature, relative humidity and global radiation. Additionally precipitation data from radar measurements in a high spatial resolution were available as an input parameter. With the use of GIS, daily spatial risk maps were created which help to identify hot spots of disease infections and simplify the interpretation of the models results (Figures 1 & 2).

In this study a new method of calculating the meteorological input parameters for simulation with GIS is demonstrated using the example of SkleroPro. The model SkleroPro calculates the risk of *Sclerotinia* stem rot (SSR) on oilseed rape (OSR). The spatial meteorological input is used to calculate the microclimate in the OSR canopy and to detect first possible infections of SSR. Finally the results were presented as spatial risk maps to provide a simplified interpretation of the models results for farmers and advisers. Moreover

SkleroPro provides a field-specific, economy-based recommendation which results in a treatment decision. With the new GIS method and the use of spatial precipitation data, local climate conditions especially convective rainfall events can be considered as a model-input.

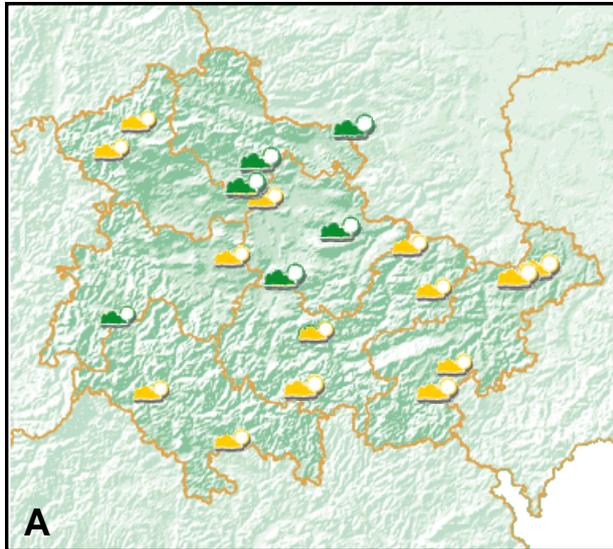


Figure 1. Former map of SkleroPro. The model results are shown at the met. station locations with coloured cloud symbols.

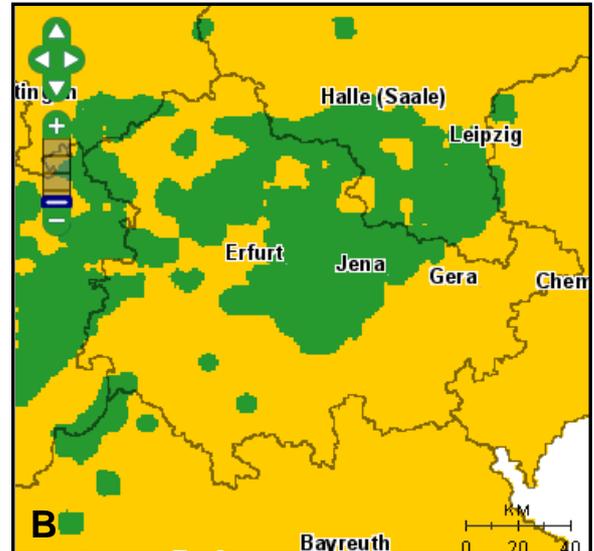


Figure 2. New map of SkleroPro. The model results are shown as a spatial risk map based on virtual met. stations with a spatial resolution of 1 km<sup>2</sup>.

## Material and methods

### *Management of met. data*

The first step to produce risk maps was data management. Therefore the met. data of about 600 automatic met. stations all over Germany provided by the German Meteorological Service (GMS) and by the Governmental Crop Protection Services (GCPS) were collected, tested on plausibility and stored in a database called AgmedaWin (Keil & Kleinhenz, 2007; Racca *et al.*, 2011). For the simulation of SkleroPro, hourly data of temperature, relative humidity, precipitation and global radiation are necessary from April to end of May .

### *Georeference of met. data*

During the next step, the met. data are georeferenced. The geodata which are identified as the basis for interpolation are latitude, longitude and elevation. For the interpolation of hourly temperature, relative humidity and global radiation, the method of multiple regression was chosen since this results in the smallest difference between measured and calculated met. data in comparison to other interpolation methods tested (Inverse Distance Weighted, Spline, Kriging). Moreover that method gave the best performance under IT-aspects. In order to store the results of the interpolation, a grid was laid out over Germany. The GCPS used about 600 met. stations to represent the agricultural area of Germany (some 200,000 km<sup>2</sup>). With the new GIS method, a grid cell had a size of one km<sup>2</sup> and after interpolation was represented by a virtual met. station.

### Precipitation

Sixteen radar stations are run by the GMS to record precipitation all over Germany. Unfortunately, the radar measurements do not record the amount of precipitation at ground level but only the signal reflected by the rain drops in the atmosphere. Consequently, the measurements only allowed calculation of an unspecific ‘precipitation intensity’. With the recently added system RADOLAN, intensity is now calibrated online with data from a comprehensive network of ombrometers (rain intensity recorded station on the ground level), using complex mathematic algorithms. As a result of the radar measurement and the RADOLAN calculation the amount of precipitation can be provided in a spatial resolution of 1 km<sup>2</sup> (Bartels, 2006).

### Weather forecast

To supplement the system, a spatial 3-day weather forecast was provided by the GMS (Cosmo-EU-data) for the parameters temperature, relative humidity, global radiation and precipitation (Deutscher Wetterdienst, 2011).

### Simulation model SkleroPro

SkleroPro is a simulation model which calculates the risk of *Sclerotinia* stem rot (SSR) on oilseed rape (OSR). It consists of two parts, a regional assessment of the disease risk and a field-specific, economy-based recommendation (treatment yes/no) (Koch, 2005; Koch *et al.*, 2007). For producing risk maps the regional disease assessment is used. Based on hourly measured temperature, relative humidity, precipitation and global radiation, the microclimate in the plant canopy is calculated starting at late-bud stage (GS 58) (Bleiholder *et al.*, 1989). The minimum conditions for stem infection by ascospores requires a temperature of 7 to 11 °C and a relative humidity of 80 to 86%. If a minimum of 23 continuous infection hours (expressed as an infection index) are achieved SkleroPro indicates that the first possible infections with SSR are possible. Figure 3 summarises all required data to produce risk maps.

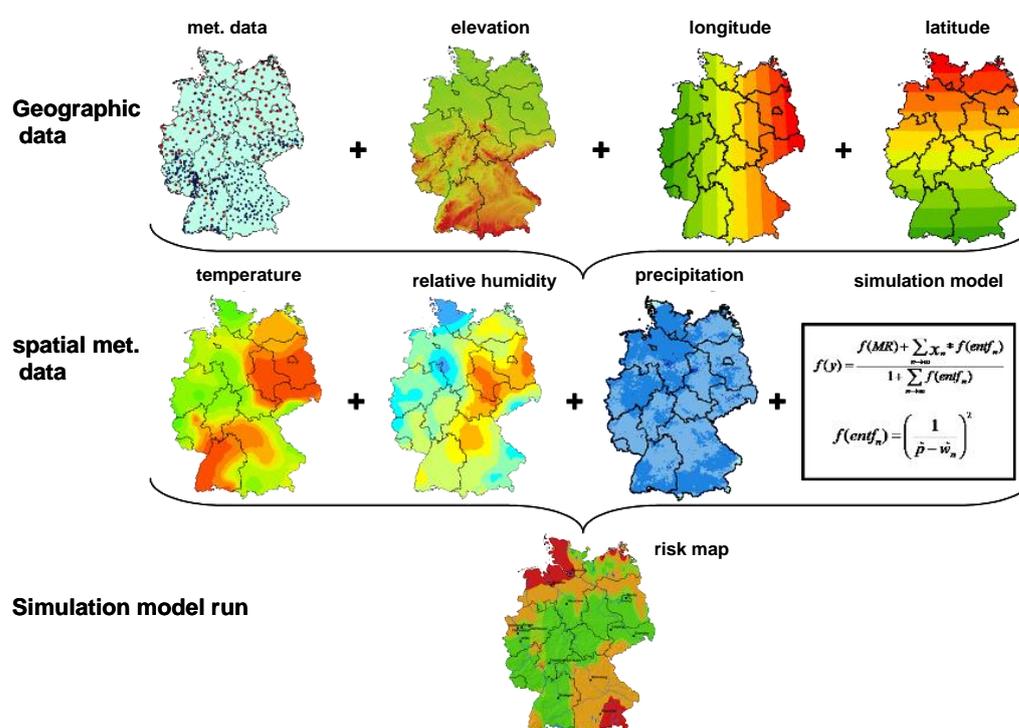


Figure 3. Process to calculate risk maps using GIS

## Results

### *Introducing spatial risk maps into practice (www.isip.de)*

The new way of presenting the results of a simulation model for plant diseases has been implemented in the Information System for Integrated Plant Production (ISIP, [www.isip.de](http://www.isip.de)). Using interpolated meteorological data in a high spatial resolution as input parameters, so-called ‘risk maps’ are drawn.

Figure 4 shows a risk map for the simulation model SkleroPro. As input parameter the period of mid-bud stage was selected. The daily updated map gave an overview about SSR hot spots in Germany and was mainly of interest to advisers. By clicking on the map, the federal state can be selected and moreover the user can zoom in to find the model results for a particular field. To see the change of prognosis, the user can scroll back to show the risk maps of up to ten days before. Additionally by scrolling forward the simulation results based on a 3-day weather forecast are presented. If the risk map turns from “green” to “yellow” the user should start field specific calculation because first infections of SSR are then possible.

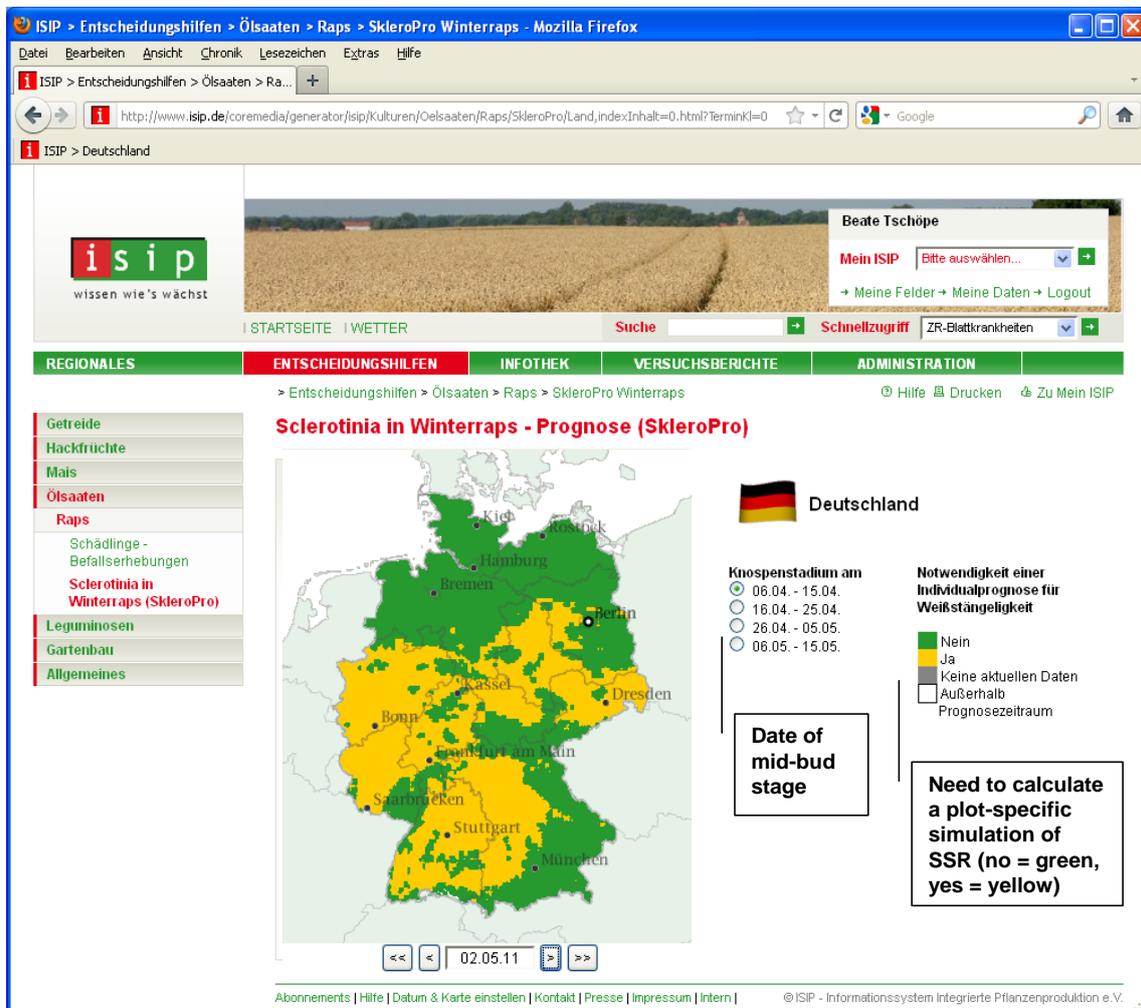


Figure 4. Risk map of SkleroPro

Besides the regional disease risk assessment, SkleroPro provides a field-specific, crop-loss-related recommendation which results in a treatment decision. With the use of interpolated met data and spatial precipitation data local climate conditions, especially convective rainfall events can be considered as a model-input. Moreover, further input data are needed for field-specific calculation: longitude and latitude of the field, crop rotation, date of mid-bud stage (GS 55), costs of spraying, expected yield and price of rapeseed (Figure 5). The result of a field specific calculation is a treatment recommendation depending on the simulated SSR infections and the cost/benefits of the treatment. In Figure 6, a diagram of SkleroPro is shown. The green curve represents the infection hours expressed as an infection index. The red curve is the summarised infection index. If the red curve exceeds the damage threshold, SkleroPro recommends a treatment. The 3-day weather forecast is also implemented in the field specific calculation. Finally the system SkleroPro is supplemented by regional adviser recommendations and a SMS- or e-mail-Service which informs growers/advisors about treatment recommendation

The screenshot shows the SkleroPro web application interface. The page title is "Sclerotinia in Winterraps - Prognose (SkleroPro)". The interface includes a navigation menu on the left with categories like "Getreide", "Hackfrüchte", "Mais", "Ölsaaten", "Raps", "Leguminosen", "Gartenbau", and "Allgemeines". The main content area features a map of the region and a form for entering field data. The form fields include:

- Neuen Prognosestandort mit Klick in die Karte oder durch Ortseingabe auswählen (with a text input for "PLZ/Ort")
- Schlagname: Niederstöcken
- Koordinaten: 9 32369825 52 4103164 RID: 197470
- Kultur: Winterraps
- Knospenstadium (BBCH 55): 2011, April, 21
- Sklerotiniaanfällige Kultur zuletzt vor: radio buttons for "Zwei Jahren", "drei Jahren", "vier oder mehr Jahren"
- Ertragserswartung: 40 dt/ha
- Preis: 43 EUR/dt (15-50 EUR/dt)
- Mittelkosten: 50 EUR/ha (25-70 EUR/ha)
- Überfahrtkosten: 10 EUR/ha (3-15 EUR/ha)

At the bottom of the map, there is a legend: "Legende / Navigation der Karte". At the bottom of the page, there are links for "Abrechnen" and "Zum Formular für Wetterstationen".

Figure 5. Input-mask of SkleroPro - field specific calculation

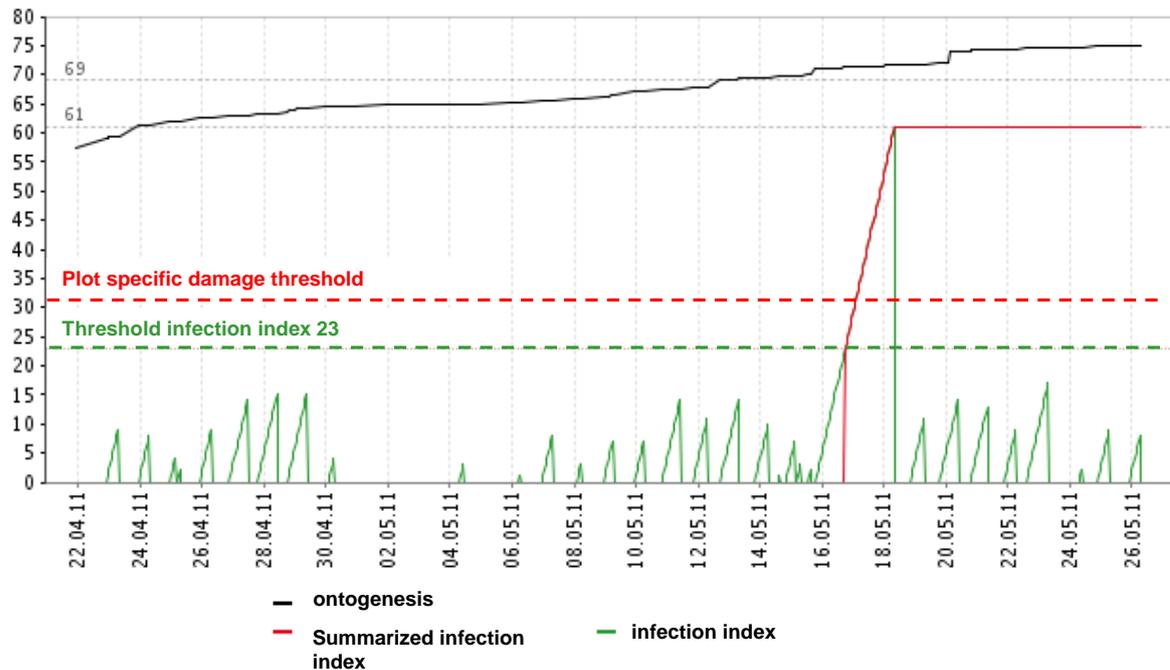


Figure 6. Output of SkleroPro-field specific calculation

## Conclusion

With the combination of simulation models for plant diseases and the weather interpolation methods based on GIS, a significant advance in advice to farmers can be provided. GIS methods and spatial precipitation data will help to produce more detailed calculations and results with higher accuracy and validity than before.

Risk maps have several advantages compared to results representing point information based on single met.stations:

- A risk map is more suitable to show hot spots for disease infections and makes the interpretation of the models results easier.
- The user does not have to choose a specific met.station, which might not even be valid for his fields.
- Local climate conditions, especially rainfall events, can be considered.
- In addition to the GIS functionalities of zooming and panning, it is possible to scroll through the maps of the last ten days and the next 3 days (based on weather forecast). This gives an excellent overview of the temporal development of the disease risk.

## Acknowledgements

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## Reducing the impact of sclerotinia disease: inoculum detection and forecasting fungicide timing in oilseed rape

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**Abstract:** *Sclerotinia* disease caused by the fungus *Sclerotinia sclerotiorum* causes significant losses in oilseed rape and other arable and vegetable crops in the UK, with infected crops providing a potential source of infection to subsequent and/or nearby susceptible crop species. Strategies for control are mainly focused on infection from the airborne spores produced when sclerotia germinate, but there is interest in targeting sclerotia in soil as well. This report focuses on improving the timing of foliar fungicide applications, but is part of a larger project on integrated control, also investigating control of the soil-borne phase, the potential for improved control from co-operation between farms, and modelling the effects of rotations. Boscalid with metconazole was applied as single sprays at yellow bud, early-, mid-, and late-flower, and also as two and three spray programmes at combinations of these times, to an oilseed rape trial at ADAS Rosemaund, Hereford, UK 2010. Two forecasting models were adapted for use and tested in-field. A spore infection model based on SkleroPro infection conditions gave approximately 90% control using 48 hr forecast alerts to guide spray timing (two sprays). A germination model predicted a late spray which was less effective. Petal sampling on four occasions during flowering indicated moderate-high inoculum according to agar plate tests. PCR tests on 24 hr Burkard spore trap samples showed that some ascospore inoculum was present earlier than detected on petals or by observations of sclerotial germination, and concentrations increased and peaked during flowering. Forecasting based on weather data may need to be combined with inoculum measurements to be more effective.

**Key words** *Sclerotinia*, oilseed rape, prediction model, inoculum

### Introduction

*Sclerotinia* disease caused by the fungus *Sclerotinia sclerotiorum* is a major problem in the UK, causing significant losses in oilseed rape and other arable and vegetable crops in the rotation, with all infected crops providing a potential source of infection to subsequent and/or nearby susceptible crop species. *Sclerotinia sclerotiorum* has two main lifecycle phases susceptible to control measures: sclerotia (resting bodies) in soil, and airborne ascospores released from apothecia produced by germinating sclerotia. Control currently relies on foliar fungicides which prevent infection by the airborne spores, but there is scope to improve the timing of fungicide applications, either by measuring the amount of airborne spore inoculum and/or by using weather data to predict when infection will occur. In addition, soil management and crop rotation measures would reduce numbers of sclerotia, which is a longer-term approach but effective when combined with foliar fungicides. This report focuses

on research to improve the timing of these fungicide applications, but is part of a larger project on integrated control of *Sclerotinia* disease in the UK, which includes work on additional fungicide timing sites, soil treatments, sclerotial inoculum production in different crops and modelling the effects of rotations and land use on the economics of *Sclerotinia* disease control.

The challenge in the UK is to improve the timing of fungicide applications for *Sclerotinia* disease and reduce the unnecessary additional treatments. Increasingly, oilseed rape crops in the UK are sprayed twice during flowering on a preventative basis but in hindsight some sprays are found to be unnecessary, and some sprays ineffective. In the absence of any other information, sprays are advised usually at early flower, and then again approximately 3 weeks later if disease pressure is high and weather conditions conducive to infection, i.e., warm and wet. Understanding the timing of release of airborne spores and their numbers could be important for targeting fungicide applications. A quantitative PCR method has been developed for direct detection of *S. sclerotiorum* spores (Rogers *et al.*, 2009), which is being tested in field experiments to determine the relationship between spore numbers and disease incidence, and indicate whether spore detection methods can be used in practice for timing fungicides. As an alternative to direct detection of spores, spore production and/or infection risk periods can be predicted indirectly using weather-based approaches. Two models have been selected for field testing: [1] a sclerotial germination model and [2] a spore infection risk model. The germination model [1] uses simple data inputs of rainfall and soil temperature, to predict when sclerotia will germinate to produce apothecia (Young *et al.*, 2008) and was developed from a simulation model for germination (Clarkson *et al.*, 2007). The spore infection risk model [2] uses the infection criteria for *Sclerotinia* based on the SkleroPro infection model (at least 23 hours at RH%  $\geq$  80%,  $> 7$  °C; assumes ascospore inoculum is not limiting; Koch *et al.*, 2007). However, the predictions of infection event dates, start time and duration are made using forecast weather data (24 and 48 hr forecasts). The infection criteria were useful in 2007 for identifying infection periods retrospectively (Gladders *et al.*, 2008), but predictions would be more helpful, because *Sclerotinia* fungicides have low curative activity.

## Material and methods

### *Fungicide timing experiment*

A replicated field experiment was established in a randomised complete block design in the Hereford area, West Midlands, drilled 31 August 2009 in a commercially grown crop of oilseed rape var. Flash, in a field with a known history of *Sclerotinia* disease. Sclerotia collected from a previous crop in the area were buried at 2 cm depth in grids on 24 October 2009, 100 sclerotia in total. Germination of sclerotia was recorded weekly from mid-April 2010. Boscalid (12% w/w) with Metconazole (5.4% w/w) (as Tectura, BASF plc) was applied at 1 l/ha, as single sprays at yellow bud, early-, mid- or late-flower (17 April, 24 April, 10 May and 22 May, respectively), and as two-spray and three-spray programmes using combinations of these times. Applications were also made according to alerts, as and when they occurred, from the two prediction models. Natural *Sclerotinia* infection developed and assessment of disease was made post flowering and immediately pre-harvest on 200 plants per plot. Incidence (% plants infected) was recorded.

### ***Forecasting models***

The date, start time and duration of infection periods, based on the 'SkleroPro' infection criteria, was calculated using 24 hr and 48 hr purchased forecast data for air temperature, rain and % relative humidity, for the Rosemaund site. Subsequently, this data was compared to actual data recorded in-field. Alerts were sent out 3 times a week to inform of any forecast infection periods, including nil predictions.

Rainfall and soil temperature (2 cm) data were recorded hourly for the field in which the oilseed rape experiment was located, from October 2009. Starting March 2010, weather data was downloaded and the germination model run weekly using data from October 2009 to the most recent; the model does not use forecasted data. The model estimates the time to 10% sclerotia germination and is based on weather records for the whole crop season which are overwritten each time a new weather download for the current season becomes available. In this way, the dates predicted for 10% sclerotia germinated may change each week from March onwards as the new seasons data is fed in the model.

### ***Inoculum detection***

Two Burkard spore traps, located at each end of the trial, were run continuously from just prior to flowering to after flowering had ended. The Burkard drums were changed weekly and sent for testing by qPCR (at Rothamsted Research), to give daily amounts of sclerotinia DNA. Petals were sampled at growth stages (GSs) 4.2, 4.5, 4.7, and 4.9, from 16 randomly selected plants in each untreated plot, from 1 flower per plot. One petal from each flower was placed on potato dextrose agar amended with 50 µl/m streptomycin sulphate, and plates were assessed after 8-10 days at room temperature for the presence of *Sclerotinia*.

## **Results and discussion**

### ***Forecasting and fungicide timing***

In addition to the four scheduled spray times, there were 2 additional spray dates based on the SkleroPro infection model. The model gave several alerts during flowering (Figure 1) using 48 hr predictions, but the earliest was considered to be at a low risk time because few flowers were open, therefore no spray was applied. A fungicide treatment was applied 4 May in response to a SkleroPro alert on 5 May towards mid-flower; the next alert was considered to be within this protection window (i.e., no spray applied), and one further spray was applied 2 June after alerts predicted at the end of flower. In untreated plots the average incidence of stem rot was moderate at 10.8%, and treatments which included yellow bud, early or mid flower sprays were effective as the key infection events during flowering were covered (Figure 1). Control using the SkleroPro model timings, which included an early-mid flower spray was good (1.13% incidence). At the end of the season, the 24 hr and 48 hr forecast data were regressed against actual data and the agreement for air temperature was good (24 hr:  $R^2 = 0.86$ ; 48 hr:  $R^2 = 0.85$ ), but for % RH was less so (24 hr:  $R^2 = 0.40$ ; 48 hr:  $R^2 = 0.37$ ). Three-day forecasts were found to be much less reliable. The infection model has potential for use by individual field sites, using in-field or purchased weather data, and will be tested in two further field seasons, at additional sites in the UK.

The sclerotial germination model final prediction for 10% germination was 31 May 2010, which was at the very end of flowering and therefore late, and gave low control (Table 1). The late prediction was unusual among the sites tested and the model will be reviewed. It is possible that if germination predictions can be improved, they could be used for individual sites or on regional basis to highlight the onset of weather conducive to production of

apothecia. There are regional differences in the onset of germination in England, with the more northerly sites tending to germinate later (Gladders *et al.*, 2011).

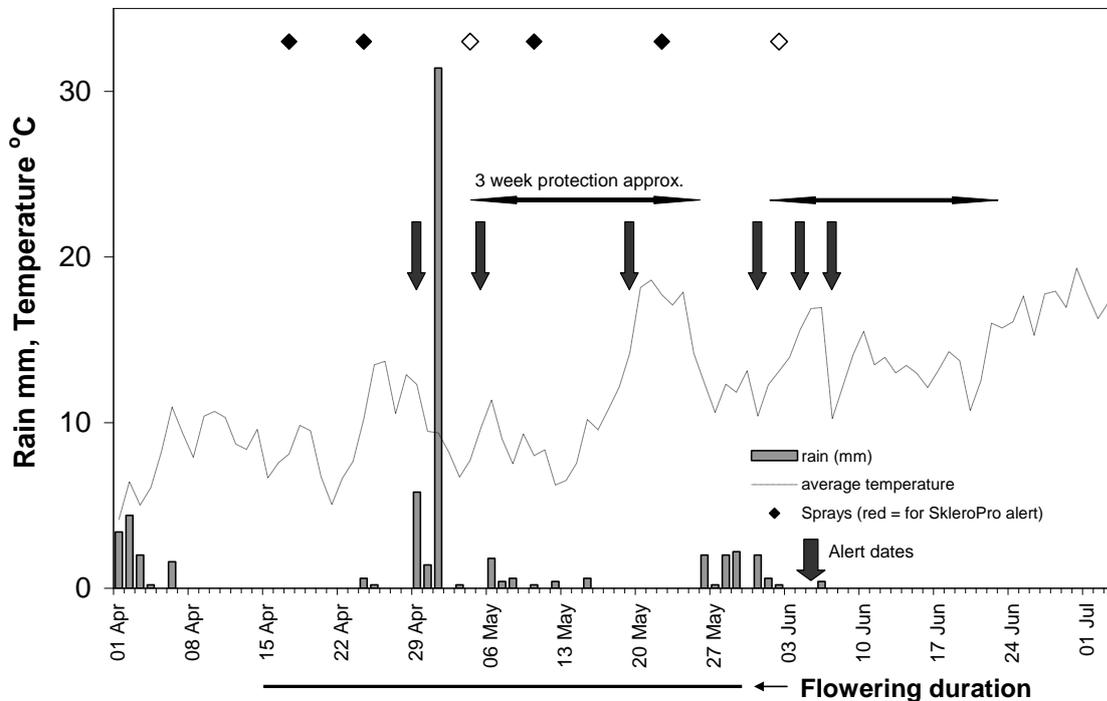


Figure 1. ADAS Rosemaund 2010, forecasting infection events and spray timing for *Sclerotinia* disease in oilseed rape. Alert dates (vertical arrows) are risk infection dates according to SkleroPro.

### ***Inoculum detection***

Petal tests by agar plating showed that inoculum was present at moderate-high levels at each of the four sample times, with 54%, 72%, 52% and 79% of petals testing positive at 20%, 50%, 70% and 90% of flowers open, respectively. In hindsight, an infection risk assessment based on petal infection would have been fairly accurate. However the agar plate method takes too long for practical purposes (8-10 days to ensure presence of *S. sclerotiorum* and not *Botrytis*), and results based on PCR test results which could be quicker to produce, will be investigated. Onset of sclerotial germination observed from sclerotia buried on-site in grids was also a good indicator at this site of spore inoculum availability at early flower. Inoculum (measured by agar plating of petals) was variable across other sites in the UK that were tested as part of this project, ranging from very low to consistently high during flowering. A combination of forecasting based on weather, and actual in-field inoculum measurements, is likely to be effective.

The key factors for the start of sclerotial germination, and therefore airborne spore production, are sufficient soil moisture, and rising temperatures. Rain events at early flower may have triggered germination, but PCR tests on Burkard spore trap samples showed that some inoculum was present in the air before sclerotial germination was observed in grids, and before the first petals were sampled (Figure 2). The initial DNA measurements were low, but the first peak coincided with the onset of germination, and in general the new germination events increased and declined in step with the peaks of DNA determined from the daily air samples

from the Burkard spore traps (Figure 2). Inoculum was produced beyond the end of flowering, which may have posed a risk for late infections. These are not as damaging to yield as early infections which tend to cause lesions which girdle the main stem low on the plant, but nonetheless may contribute to yield loss.

The results from the ADAS Rosemaund site and other sites within this project suggest that forecasting *Sclerotinia* risk based on weather data could be effective, but will probably need to be combined with measures of spore levels and timing, as inoculum pressure appears to vary between UK sites. The potential to combine improved targeting of foliar fungicides with longer term soil management, rotational and land-use approaches is being investigated within this project. For example, initial experiments in 2010 with different susceptible crops suggest that some crops produce many more sclerotia than others, and this may need to be taken into account when assessing infection risk from field history and adjacent fields.

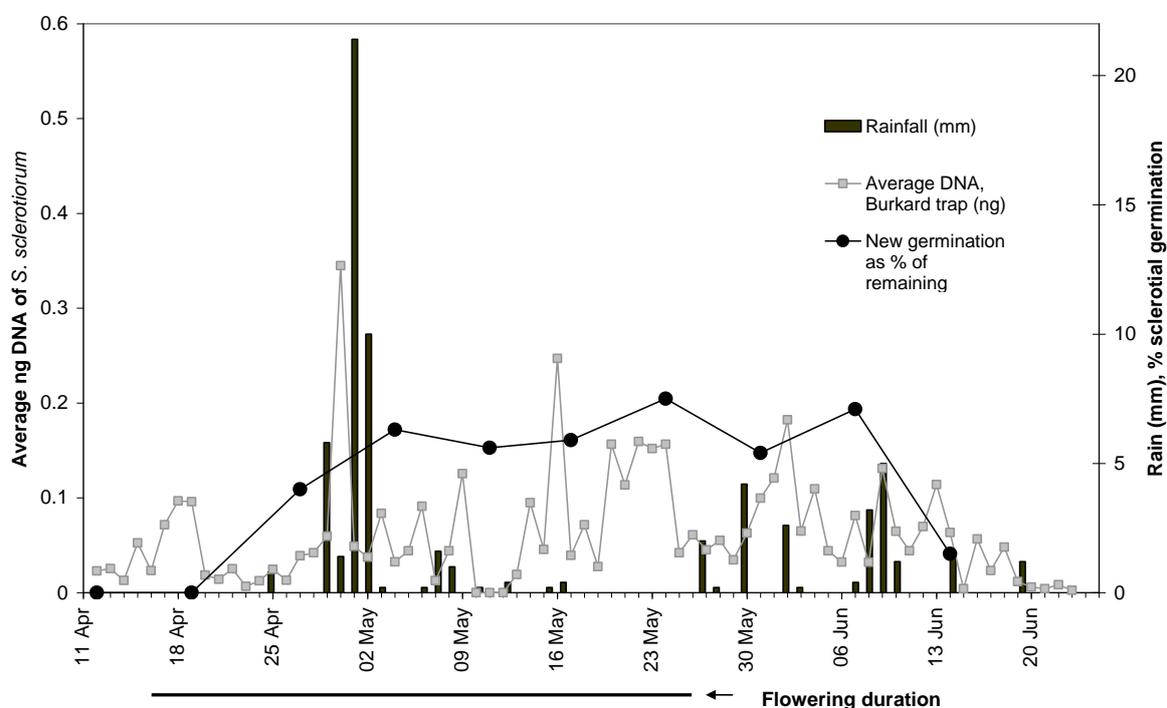


Figure 2. ADAS Rosemaund 2010, sclerotial germination, *Sclerotinia* DNA from PCR tests (Burkard spore trap samples) and rain during flowering of oilseed rape.

## Acknowledgements

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## Studies on the optimal timing of fungicide application against *Sclerotinia* stem rot in southern Poland

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**Abstract:** *Sclerotinia* stem rot caused by *Sclerotinia sclerotiorum* is a major pathogen of oilseed rape (OSR) causing greatly decreased seed yield. Sclerotia of the fungus reside in the soil and germinate to form apothecia, which then release airborne ascospores. Spores usually infect plants via petals, and are later deposited on leaves or stems. Petals of OSR are good sources of nutrition for the fungus. In Poland the control of the disease relies on application of fungicide, but choosing appropriate timing of sprays and working out the necessity of treatment based on real levels of primary inoculum is still a challenge. To address this problem a petal test using media with pH indicators that change colour when they are acidified with oxalic acid – the main metabolite of *S. sclerotiorum* – has been implemented. The main aim of this study was to determine the effect of fungicide application in different times during flowering stage of OSR and to compare the results of petal contamination with the subsequent infection of OSR plants. The experiments were done in two seasons: 2009/2010 and 2010/2011. The field was situated in Glubczyce, located in southern Poland. The winter OSR hybrid PR46W31 (Pioneer Hi-Bred) was tested. Fungicide treatments were done at weekly intervals from late April to mid July, with Acanto SC 250 containing 250 g picoxystrobin per 1 l of the fungicide. The stem canker symptoms before harvest were evaluated according to a scale from 0 to 9, where 0 was no visible symptoms and 9 was a plant totally damaged by the disease. Each experiment variant was also evaluated according to the following characters: seed yield at 90% of dry matter content, mass of thousand seeds (MTS), the percentage of oil in seeds, the content of proteins, the content of acid and neutral detergent fibers, the content of five damaging glucosinolates: glucobrassicinapin, progoitrin, napoleiferin, glucobrassicin and 4-OH glucobrassicin. Each season the petals were chosen randomly from an untreated variant, six times during the flowering stage. Scoring of fungal colonies was done based on media discoloration followed by sclerotia formation. Time of fungicide application had a statistically significant impact on the effectiveness of chemical protection of OSR against *Sclerotinia* stem rot. The highest disease incidence was observed in the unsprayed variant, with 25% of infected plants in 2010 and 50% in 2011. Application of fungicide reduced disease incidence. The percentage of infected plants depended on the time of fungicide application. It ranged from 4% (spray on 26 April) to 12.7% (28 May) in 2010 and from 10% (4 May) to 27.3% (15 July) in 2011. The comparison of the availability of primary inoculum in the air (up to 100% of petals infected with *S. sclerotiorum*) and the subsequent plant infection have demonstrated that the infection of OSR plants was much smaller than the infection of petals, indicating that most of the infected petals did not initiate the disease. The proportion of petals that started the disease greatly depended on weather conditions, mainly rainfall and humidity. This correlation was highly significant, suggesting that the petal test supplemented with a relatively simple analysis of rainfall may serve as a good tool for predicting the incidence and severity of *Sclerotinia* stem rot of OSR. Low percentages of infected plants due to early fungicide sprays suggest that farmers growing OSR in high risk areas should protect the plants of OSR at early flowering stage.

**Key words:** *Sclerotinia sclerotiorum*; oilseed rape; petal test; weather data



*Verticillium*



## Studies on the improvement of winter oilseed rape resistance to *Verticillium longisporum*

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**Abstract:** The soil-borne fungal pathogen *Verticillium longisporum* (VL) causes premature ripening on oilseed rape and can lead to yield damage under favorable conditions. Melanized resting structures of the pathogen, microsclerotia, can persist for several years and can therefore cause a long-term contamination of the soil. Since there are no effective fungicides available, breeding for resistance is one of the most efficient measures to control the disease. Conventional screening of field-grown winter oilseed rape (WOSR) for evaluation of resistance to VL is performed by visual detection of microsclerotia in OSR stubbles. Although this method allows for a rough estimate of the infestation level, a late detection of the pathogen together with interference by the plants' ripening stage limits the applicability of the method. Here, we report on the development of a quantitative real-time PCR method to detect and quantify VL in field-grown OSR. Two primer pairs targeting distinct gene loci in *V. longisporum* were evaluated for their specificity and sensitivity in detection of VL DNA. The primers targeting the ITS (Internal transcribed spacer) region showed high sensitivity and were specific for *Verticillium* species, but not for VL isolates. Primers targeting the beta-tubulin-region were considerable less sensitive, while showing high specificity for most of the tested VL isolates. Application of ITS-based qPCR permitted detection of the pathogen before symptom expression in stems of four field grown WOSR cultivars with varying susceptibility in the season 2008/09. The fungal DNA concentrations in the four cultivars correlated with the stubble and greenhouse screening when grouped into resistance classes. This method provides a tool for classification of resistant genotypes for integration in breeding of VL resistant elite cultivars. Furthermore, we studied ultrastructural changes in the hypocotyl of *B. napus* after infection with VL by transmission electron microscopy. Upon infection, ultrastructural changes of the vascular tissue, such as formation of occlusions and secondary cell wall covering occurred in both susceptible and resistant plants. This suggests that resistance is rather based on quantitative differences in the vascular tissue.

**Key words:** *Brassica napus*; premature ripening; PCR detection; quantitative PCR



## Response of oilseed rape (*Brassica napus* L.) to combined effects of drought stress and *Verticillium longisporum* infestation

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**Abstract:** Oilseed rape (OSR) is one of the economically most important oil crops in the world. It is grown for several purposes including production of vegetable oil, animal feed, and other food and non-food products. *Verticillium longisporum* is among the important fungal pathogens that attack OSR plants. This pathogen is a host specific fungus and it mainly attacks cruciferous plants and causes premature senescence and ripening which ultimately leads to severe yield losses. Artificially inoculated plants under greenhouse condition also show additional symptoms like foliar chlorosis, severe stunting of shoots and reduction of root length. When diseased plants are harvested, the microsclerotia are released in the soil where the pathogen may survive in dormancy for several years. This makes the fungus very difficult to control with chemicals and other means of control such as crop rotation. As a result, very limited options are available to control the disease and studies suggest that plant resistance remains the preferred strategy. However, currently this method is not yet an option for producers since very limited studies regarding development of resistant varieties and knowledge on mechanisms of resistance are available. Breeding efforts during the past few years that aimed at developing resistance genotypes through crossing appeared to be successful and at present OSR double haploid (DH) lines and genotypes that show different levels of resistance towards *V. longisporum* are available. Similar studies also showed that significantly higher build-up of vascular occlusions, cell wall-bound phenolics and lignin are the major resistance mechanisms responsible for blocking further colonization of plant tissue with *V. longisporum* towards the shoot system of the plant after root infection. However, the negative consequence of these resistance mechanisms on translocation of water and essential nutrients in the plant system which may ultimately affect the quality and quantity of yield particularly under drought conditions are not known. In the present study, we investigated the combined effects of *V. longisporum* and drought stress on disease development, morphological and physiological attributes and yield performance of two oilseed rape genotypes.

An experiment consisting of three factors namely, genotype, disease and water stress factors was conducted and several disease, agronomic, physiological and biochemical characteristics that best explain resistance of rapeseed cultivars towards *V. longisporum* and drought stress were assessed and analysed. The study was conducted under greenhouse condition and disease index, agronomic and yield parameters were assessed using standard methods. A WALZ portable photosynthesis system was used to monitor stomatal conductance, photosynthesis and transpiration rates. Leaf proline content and fungal biomass in hypocotyl tissue were determined by spectrophotometer and qPCR, respectively.

Our results showed that *V. longisporum* caused severe disease and reduction of plant biomass in the susceptible cultivar (up to 36%). Moreover, we observed that drought stress has a significant effect on agronomic and yield parameters and fungal growth. It also slightly induced proline accumulation in the tolerant genotype SEM. However, addition of drought

stress to inoculated plants did not significantly affect disease, physiological and yield parameters indicating that the above mentioned resistance mechanisms of OSR do not interfere with translocation of water and nutrients. In addition, the results from the present study provided evidence that *V. longisporum* resistance of OSR genotypes against *V. longisporum* is stable even under conditions where there is drought stress. Further investigations on physiological, histological and molecular genetic characteristics that may be affected by these two stress factors are required.

**Key words:** *Verticillium longisporum*, drought stress, oilseed rape, *Brassica napus*

**Clubroot**



## Ten years experience with the clubroot resistant cultivar ‘Mendel’: performance and perspectives

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**Abstract:** The clubroot resistant oilseed rape cultivar ‘Mendel’ has been released in 2001 to the European seed market. Since its introduction ‘Mendel’ has an average market share of 1 to 2% in European winter oilseed rape seed sales. It is preferably used in cropping areas that have a significant clubroot risk, such as the eastern parts of Schleswig-Holstein, where its market share is estimated to exceed 15%.

‘Mendel’ is a hybrid cultivar which has been bred using a resynthesised *Brassica napus* form as resistance donor. The resynthesised form originated from a cross between a clubroot resistant curly kale (*B. oleracea*) and a clubroot resistant stubble turnip (*B. rapa*), and had been shown to possess a broad spectrum resistance. While the stubble turnip parent is expected to carry 3 dominant resistance genes, ‘Mendel’ has been shown to have at least one dominant resistance gene.

The clubroot resistance of ‘Mendel’ is acting in a race-specific manner and compatible races have been present already before its introduction, however, the frequency of compatible isolates has been low. Monitoring for compatible races by the breeders has focused on farmer reports about clubroot in ‘Mendel’ crops and the confirmation of the compatibility of the local race in greenhouse assays. In many cases the local race could be classified as still incompatible. Compatible races occurred more frequent in certain areas such as the north-east of Mecklenburg-Vorpommern or parts of Westphalia. So far there is no indication for a close relation between the frequency of ‘Mendel’ within the rotation and the frequency of compatible isolates.

‘Mendel’ has a yield potential which is comparable to other hybrid cultivars from the same breeding period. Breeding efforts should lead to the release of new clubroot resistant cultivars which are comparable in yield to recent cultivars and have a broader resistance spectrum in the near future.

**Key words:** clubroot; oilseed rape; resistance; races; geographic distribution

## **Virulence analysis of *Plasmodiophora brassicae* derived from different locations of the main European oilseed rape growing regions**

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**Abstract:** Clubroot caused by the obligate biotrophic protist *Plasmodiophora brassicae* is a serious soil-borne disease of cruciferous crops. It causes galls to form on roots leading to premature death of the plant. The organism remains in the soil as resting spores which can survive for up to 20 years. Therefore, there are no economically reasonable control measures once a field has been infested. The hot spots of clubroot infestation in Europe are mainly located in Scotland, England, France and northern Germany. Currently, due to an increase of oilseed rape cropping within the last decades the number of contaminated fields detected all over Europe has increased. There are numerous populations and races, respectively, of *P. brassicae* with differences in pathogenicity causing different symptoms on the host plant. For this reason resistance breeding is difficult.

To get more detailed information on the occurrence and the virulence of *P. brassicae* and its implications for agricultural production, samples of infected plant material were taken from locations all over the main oilseed rape growing regions in Europe. Currently the collection contains samples from the United Kingdom, France, Denmark, Poland, the Czech Republic and mainly from numerous locations in Germany. These samples were actually analysed under greenhouse conditions by using artificial inoculation and performing optical ratings of disease symptoms. The European Clubroot Differential Set (ECD) consisting of 15 genotypes which are subdivided into 5 lines each from 3 different species i.e. *Brassica rapa*, *B. napus* and *B. oleracea* and the INRA differential set (Hôte différentiel de l'INRA) composed of three *B. napus* genotypes, i.e. 'Nevin', 'Brutor' and 'Wilhelmsburger', were used for these tests, respectively.

First results of these analyses produced evidence for different pathotypes present across Europe. The benefit of this geographic monitoring will be on the one hand general information about the dimension of clubroot disease in Europe and on the other hand the appearance of specific pathotypes. This is a prerequisite for an efficient breeding for resistance as up to now only one race specific resistance is incorporated in adapted cultivars.

**Key words:** clubroot; population; races; sampling

## **Influence of soil moisture and temperature on the infection of oilseed rape with *Plasmodiophora brassicae***

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**Abstract:** The effect of soil moisture and temperature on the effectiveness of spore infection of oilseed rape with the soilborne pathogen *Plasmodiophora brassicae* was studied in two trials under greenhouse conditions. In both trials the rape seed plants were cultivated in a soil-sand-turf mix under a consistent pH-value of 5.4. Ten days after sowing the rape seed was inoculated with a 2 ml spore suspension ( $1 \times 10^7$  spores/ml) placed on each root neck. In the temperature trial four different variants (10, 15, 20 and 25 °C) were adjusted at consistent soil moisture of 100% of the water holding capacity. In the soil moisture trial three different variants were adjusted (100, 80 und 60% of the water holding capacity) at consistent temperature of 20 °C. The soil moisture was controlled daily. In both greenhouse trials the severity of typical symptoms of *P. brassicae* infection on the roots of 25 plants was visually examined once a week. Disease symptoms could be observed in all temperature variants five weeks after inoculation. The severity of *P. brassicae* infection decreased from high to low temperature. The results of the second trial indicate a stronger effect of soil moisture on the severity of *P. brassicae* infection than temperature.

**Key words:** clubroot; oilseed rape

