

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

BIANNUAL MEETING

# Prospects and Progress for Sustainable Oilseed Crop Protection

September 7–9, 2016  
Tartu, Estonia



Cover: *Brassica napus* – Franz Eugen Köhler, *Köhler's Medizinal-Pflanzen* (1897)

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# Prospects and Progress for Sustainable Oilseed Crop Protection

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# Schedule of the Working Group Meeting

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## Programme Overview

Day	Event	Start	End	Room
<b>7<sup>th</sup> September, Wednesday</b>	Registration	08:30	10:00	Entry Hall
	Welcome	10:00	10:45	1A5
	Introduction to IOBC-WPRS	10:45	11:00	1A5
	Coffee break	11:00	11:30	Entry Hall
	Joint Session 1	11:30	12:30	1A5
	Lunch	12:30	14:00	Canteen
	Entomology Session 1 (a)	14:00	15:20	D239
	Plant Pathology Session 1	14:00	15:20	2A1
	Coffee break	15:20	16:00	Entry Hall
	Poster session	16:00	17:00	Entry Hall
<b>8<sup>th</sup> September, Thursday</b>	Joint session 2	09:00	10:30	1A5
	Coffee break	10:30	11:00	Entry Hall
	Entomology Session 1 (b)	11:00	12:30	D239
	Plant Pathology Session 2	11:00	12:30	2A1
	Lunch	12:30	14:00	Canteen
	Entomology Session 2	14:00	15:30	D239
	Plant Pathology Session 3	14:00	15:30	2A1
	Coffee break	15:20	16:00	Entry Hall
	Entomology Session 3	16:00	17:15	D239
	Plant Pathology Session 3	16:00	17:15	2A1
Conference dinner at Mooste	17:15	22:30		
<b>9<sup>th</sup> September, Friday</b>	Entomology Session 4	09:00	10:40	D239
	Plant Pathology Session 4	09:00	10:40	2A1
	Coffee break	10:40	11:00	Entry Hall
	Joint Session 3	11:00	13:00	1A5
	Lunch and departure	13:00	15:00	

## 7<sup>th</sup> September, Wednesday

8:30-10:00	Registration <b>Entry Hall</b> (Kreutzwaldi 5)	
<b>JOINT SESSION 1</b>	<b>Chair: Małgorzata Jędrzycka</b>	<b>Room: 1A5</b>
10:00	Welcome – Małgorzata Jędrzycka & Samantha M. Cook	
10:15	Welcome – Eve Veromann & Aret Vooremäe	
10:45	Giselher Grabenweger Introduction to IOBC-WPRS	
<b>11:00–11:30</b>	<b>Coffee break</b>	
11:30	Nils Conrad	Impact of different insecticides sprayed in autumn or treated to winter oilseed rape seeds on the number of <i>Psylliodes chrysocephala</i> larvae and TuY virus infection p. 10
12:00	Robert Malinowski	<i>Arabidopsis thaliana</i> as a model plant for studying plant pathogen interactions during <i>Plasmodiophora brassicae</i> infection p. 11
<b>12:30–14:00</b>	<b>LUNCH</b>	
<b>Entomology Session 1 (a)</b>	<b>Insecticides: need, efficacy and side effects</b>	<b>Room: D239</b>
	<b>Chair: Samantha M. Cook</b>	
14:00	Samantha M. Cook	Introduction to Entomology sessions
14:10	Maria Zhang	Arthropod pest control for UK oilseed rape – expert opinions on insecticide efficacies, side effects and alternatives p. 18
14:30	Jean-Pierre Jansen	Field trials to assess the short-term and long-term effects of several insecticides used to control the pollen beetle on parasitic hymenoptera in oilseed rape p. 19
14:50	Udo Heimbach	Pyrethroid resistance of insect pests in oilseed rape in Germany since 2005 p. 20
15:10	- - - - -	Discussion
<b>15:20–16:00</b>	<b>Coffee break</b>	
<b>16:00–17:00</b>	<b>Poster Session</b>	
<b>Plant Pathology Session 1</b>	<b>Resistance to blackleg</b>	<b>Room: 2A1</b>
	<b>Chair: Nazanin Zamani-Noor</b>	
14:00	Gosia Jedryczka	Introduction to Pathology section
14:10	Birger Koopmann	Assessment of the efficiency of major resistance genes against blackleg of oilseed rape in Germany p. 54
14:30	Janetta Niemann	Resistance to stem canker ( <i>Leptosphaeria</i> spp.) in interspecific <i>Brassica</i> hybrids and rapeseed ( <i>Brassica napus</i> L.) cultivars p. 55
14:50	Lenka Burketová	Collagen and keratin hydrolyzates induce resistance against <i>Leptosphaeria maculans</i> in oilseed rape p. 56
15:10	- - - - -	Discussion
<b>15:20–16:00</b>	<b>Coffee break</b>	
<b>16:00–17:00</b>	<b>Poster Session</b>	

## 7<sup>th</sup> September, Wednesday

16:00-17:00

POSTER SESSION		Entry Hall
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Udo Heimbach, Meike Brandes, Johannes Hausmann & Bernd Ulber	Effects of conventional and dropleg insecticide application techniques on pests during flowering of oilseed rape	p. 26
Duncan J. Coston, Simon G. Potts, Tom Breeze, Linda M. Field & Samantha M. Cook	Implications of the neonicotinoid restriction on oilseed rape pest control, pollination and productivity	p. 27
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Jürgen Junk & Michael Eickermann	High Performing Computer as an efficient tool for forecasting pest insect activity	p. 35
Nils Conrad, Meike Brandes & Udo Heimbach	Adults of <i>Psylliodes chrysocephala</i> in different types of yellow water traps in winter oilseed rape	p. 37
Vojtěch Hlavjenka, Marek Seidenglanz & Jaroslav Šafář	Spatio-temporal distribution and association of cabbage stem weevil ( <i>Ceutorhynchus pallidactylus</i> Marsham, 1802) and pollen beetle ( <i>Meligethes aeneus</i> Fabricius, 1775) in winter oilseed rape	p. 38
Nils Conrad, Meike Brandes & Udo Heimbach	Automatic extraction of <i>Psylliodes chrysocephala</i> larvae versus sorting by hand	p. 39
Chris Shortall, Samantha M. Cook, Alice Mauchline, Julian Park & James Bell	Investigating the temporal and spatial ecology of the pollen beetle <i>Meligethes aeneus</i>	p. 40
Gabriella Kovács, Riina Kaasik, Kaia Treier, Anne Luik & Eve Veromann	Do different field bordering elements affect cabbage seed weevil damage and its parasitism rate differently in winter oilseed rape?	p. 46
Henrike Hennies, Katharina Lohaus & Bernd Ulber	Screening of <i>Brassica napus</i> , <i>Sinapis alba</i> and introgression lines for antixenotic resistance to oviposition by cabbage root fly ( <i>Delia radicum</i> L.)	p. 52
Andrzej Brachaczek, Witold Dzitkowski & Joanna Kaczmarek	The comparison of different nutrition and growth stimulation programs on fluorescence of chlorophyll a and gas exchange efficiency in leaves of oilseed rape	p. 70
Nazanin Zamani Noor	Variability in fungicide sensitivity of <i>Leptosphaeria maculans</i> and <i>L. biglobosa</i> , the causal agents of blackleg disease in oilseed rape	p. 76
Karolina Stefanowicz & Robert Malinowski	Proteomic approach to study cell wall changes occurring within host plant during clubroot infection	p. 77

## 8<sup>th</sup> September, Thursday

<b>JOINT SESSION 2</b>		<b>Chair: Samantha M. Cook</b>	<b>Room: 1A5</b>
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9:30	Cezary Tkaczuk	The potential of entomopathogenic fungi in biological control of oilseed rape pests	p. 13
10:00	Deborah Kaiser	Biological control of pollen beetles with the entomopathogenic fungus <i>Beauveria bassiana</i> – the tricky path to an efficient formulation	p. 14
<b>10:30–11:00</b>		<b>Coffee break</b>	
<b>Entomology Session 1 (b)</b>		<b>Insecticides: need, efficacy &amp; side effects (continued)</b>	<b>Room: D239</b>
		<b>Chair: Michael Eickermann</b>	
11:00	Celine Robert & Laurent Ruck	Pyrethroid resistance in cabbage stem flea beetle ( <i>Psylliodes chrysocephala</i> ) and rape winter stem weevil ( <i>Ceutorhynchus picipitarsis</i> ) populations in France	p. 21
11:20	Marek Seidenglanz	Correlations between susceptibilities to lambda-cyhalothrin and chlorpyrifos-ethyl with respect to thiacloprid in Czech populations of <i>Meligethes aeneus</i>	p. 22
11:40	Meike Brandes	Effects of thiacloprid on population dynamics of pollen beetles in field studies 2013–2015	p. 23
12:00	Mona Jahani	First data on RNAi to control pollen beetles	p. 24
12:20	- - - - -	Discussion	
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<b>Entomology Session 2</b>		<b>Improving decision support for IPM</b>	<b>Room: D239</b>
		<b>Chair: Eve Veromann</b>	
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14:40	Michael Eickermann	Too hot to handle? – Impact of winter temperature on populations of stem-mining pest insects	p. 33
15:00	Samantha M. Cook	How can decision support system forecasts improve management of pollen beetle and cabbage stem flea beetle in oilseed rape?	p. 34
15:20	- - - - -	Discussion	
<b>15:30–16:00</b>		<b>Coffee break</b>	
<b>Entomology Session 3</b>		<b>Delivering effective biocontrol of OSR pests via natural enemies</b>	<b>D239</b>
		<b>Chair: Bernd Ulber</b>	
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16:40	Amandine Juhel	Variation in abundance of pollen beetle, <i>Meligethes aeneus</i> and its parasitoid, <i>Tersilochus heterocerus</i> in oilseed rape in relation to proximity to woodlands, grasslands and other oilseed rape fields	p. 45
17:00	- - - - -	Discussion	
<b>17:15–22:30</b>		<b>Bus transfer to Mooste Distillery (<a href="http://www.viinavabrik.ee/distillery">www.viinavabrik.ee/distillery</a>), guided tour, dance and conference dinner</b>	



## 8<sup>th</sup> September, Thursday

<b>JOINT SESSION 2</b>		<b>Chair: Samantha M. Cook</b>	<b>Room: 1A5</b>
9:00	Julie Smith	Quantifying the non-fungicidal effects of boscalid dimoxystrobin co-formulation in winter oilseed rape	p. 12
9:30	Cezary Tkaczuk	The potential of entomopathogenic fungi in biological control of oilseed rape pests	p. 13
10:00	Deborah Kaiser	Biological control of pollen beetles with the entomopathogenic fungus <i>Beauveria bassiana</i> – the tricky path to an efficient formulation	p. 14
<b>10:30–11:00</b>		<b>Coffee break</b>	
<b>Plant Pathology Session 2</b>		<b>Blackleg distribution, severity and chemical control</b>	<b>Room: 2A1</b>
		<b>Chair: Lenka Burketova</b>	
11:00	Lakshmi Harika Gajula	Identification of new virulent races of <i>Leptosphaeria maculans</i> populations on oilseed rape in the UK	p. 58
11:20	Katherine Noel	Temperature sensitivity of <i>Brassica napus</i> resistance against <i>Leptosphaeria maculans</i>	p. 60
11:40	Jana Mazáková	Country-wide and temporal distribution of pathogens associated with phoma stem canker in the Czech Republic	p. 61
12:00	Asna Javaid	Decreasing the risk of severe phoma stem canker caused by <i>Leptosphaeria biglobosa</i> on winter oilseed rape	p. 62
12:20	Nazanin Zamani Noor	The effects of different plant growth regulators and fungicides on Phoma stem canker, growth parameters and the yield of winter oilseed rape	p. 62
<b>12:30–14:00</b>		<b>LUNCH</b>	
<b>Plant Pathology Session 3</b>		<b>Non-chemical control of diseases</b>	<b>Room: 2A1</b>
		<b>Chair: Birgen Koopmann</b>	
14:00	Małgorzata Jędryczka	The potential of Trichoderma strains for control of stem canker of brassicas ( <i>Leptosphaeria</i> spp.)	p. 64
14:20	Agnieszka Perek	The influence of antagonistic fungi on the growth of <i>Sclerotinia sclerotiorum</i>	p. 65
14:40	Agnieszka Mączyńska	The influence of crop rotation and the time of application of fungicides on the occurrence of perpetrators diseases in winter oilseed rape	p. 66
15:00	Marek Korbas	The effect of cultivation systems and crop rotation on the occurrence of weeds and diseases in oilseed rape	p. 67
15:20	- - - - -	Discussion	
<b>15:30–16:00</b>		<b>Coffee break</b>	
16:00	Faye Ritchie	Integrated management strategies for controlling light leaf spot ( <i>Pyrenopeziza brassicae</i> ) in winter oilseed rape	p. 68
16:20	Annette Penaud	SDHI resistance in French populations of <i>Sclerotinia sclerotiorum</i> and its management	p. 69
16:40	Annette Penaud	Pasmo: observations of pseudothecia of <i>Mycosphaerella linicola</i> on linseed stubble	p. 69
17:00	- - - - -	Discussion	
<b>17:15–22:30</b>		<b>Bus transfer to Mooste Distillery (<a href="http://www.viinavabrik.ee/distillery">www.viinavabrik.ee/distillery</a>), guided tour, dance and conference dinner</b>	

## 9th September, Friday

<b>Entomology Session 4</b>		<b>Breeding for resistance to insect pests for IPM strategies in OSR</b>	<b>D239</b>
		<b>Chair: Udo Heimbach</b>	
9:00	Anne-Marie Cortesero	Screening different varieties of oilseed rape for sources of resistance against insects	p. 48
9:20	Maxime Hervé	Potential for oilseed rape resistance for pollen beetle control	p. 48
9:40	Gaëtan Seimandi Corda	Testing genotype susceptibility to insect pests: an example from the oilseed rape – pollen beetle interaction	p. 49
10:00	Bernd Ulber	Semi-field and laboratory methods to screen oilseed rape genotypes for resistance to pollen beetles ( <i>Meligethes aeneus</i> F.)	p. 50
10:20	Katharina Lohaus	Screening of introgression lines for antixenotic and antibiotic mechanisms of resistance to cabbage seed weevil ( <i>Ceutorhynchus obstrictus</i> Marsham)	p. 51
<b>10:40-11:00</b>		<b>Coffee break</b>	
<b>Plant Pathology Session 4</b>		<b>Clubroot of oilseed rape</b>	<b>Room: 2A1</b>
		<b>Chair: Henrik Stotz</b>	
9:00	Veronica Řičařová	<i>Plasmodiophora brassicae</i> Wor. on winter oilseed rape in the Czech Republic	p. 72
9:20	Joanna Kaczmarek	Incidence of <i>Plasmodiophora brassicae</i> and the composition of its pathotypes in Poland	p. 73
9:40	Nazanin Zamani Noor	Suppression of <i>Plasmodiophora brassicae</i> , an emerging pathogen of German oilseed rape crop, with soil amendments	p. 74
10:00	Marcin Olszak	<i>Plasmodiophora brassicae</i> infection – usurping the host molecular regulatory networks for feeding site formation	p. 75
10:20	- - - - -	Discussion	
<b>10:40-11:00</b>		<b>Coffee break</b>	
<b>JOINT SESSION 3</b>		<b>Chair: Eve Veromann</b>	<b>Room: 1A5</b>
11:00	Samantha M. Cook & Małgorzata Jędrzycka	Summary of Entomology and Pathology Sessions	
11:30	Ivan Juran & Małgorzata Jędrzycka	Next meetings	
11:50	Małgorzata Jędrzycka	Introduction to EU discussion	
12:00	Małgorzata Jędrzycka	EU project discussion	
<b>13:00-15:00</b>		<b>LUNCH and departure</b>	

## JOINT SESSION General papers

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

Nils Conrad, Anna Köneke, Meike Brandes & Udo Heimbach

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**Abstract:** The influence of different insecticides applied to winter oilseed rape in autumn on cabbage stem flea beetles, aphids and virus infestation was investigated. In 2015 the effects of the seed treatments Elado and Fortenza Force on larvae of cabbage stem flea beetle were very limited, but

adult beetles migrated into the field very late. Spraying of a pyrethroid (Karate Zeon) in autumn reduced the number of larvae by up to 80 %. The aphid and virus infestation were significantly reduced only by using Elado as seed treatment.

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

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

Robert Malinowski

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**Abstract:** *Plasmodiophora brassicae* is a biotrophic pathogenic protist infecting plants mainly from the *Brassicaceae* family. Its life cycle is tightly linked to soil and water environments. Resting spores of this pathogen can survive in the soil for many years and germinate once optimal conditions for infection occur. The first step of infection is mediated by haploid zoospores invading root hairs of the host plant. Inside the plant the pathogen develops multinucleate zoosporangia that produce plasmodia which are released into the soil and act as a source of secondary infection. Secondary zoospores invade the underground parts of a plant and develop into motile secondary plasmodia which are responsible for further disease spread and progression within the plant. Early stages of secondary infection are accompanied by massive changes in the host metabolism leading to establishment of a new, pathogen-oriented, physiological sink. As a consequence of these changes, serious hyperplasia followed by hypertrophy is observed. This leads to the development of galls on the underground parts of infected plants. During later stages of gall development giant cells are formed. These cells are the site of spore maturation. Once spores are mature, disintegration of the underground parts occurs and spores are released into the soil. Clearly, the completion of the entire life cycle by the pathogen

is accompanied by complex reprogramming of the host. A detailed understanding of this reprogramming at the cellular and molecular levels can help to reduce the negative impact of *P. brassicae* on plant yield. Here we would like to discuss the usefulness of *Arabidopsis thaliana* as a model plant for studying complex host reprogramming by *P. brassicae*. *Arabidopsis thaliana* is a plant whose cellular and molecular regulation is well described. Due to the certain features of roots and hypocotyl *Arabidopsis* is a perfect model to localise and assign particular cellular changes to known molecular networks governing processes like cell proliferation, growth or differentiation. In our group we have developed a model describing how *P. brassicae* modulates certain cellular processes in host plants in order to usurp their nutrition and use it as a site for multiplication. The second advantage of *A. thaliana* is the possibility to use a wide range of molecular biology tools and resources which help to interpret each step of the plant-pathogen interaction. At the moment we are set up to study the infection from the very early to late stages of its progress. We recognise that certain significant differences between our model and crop plants such as oilseed rape do arise, therefore an opportunity to discuss these with oilseed rape experts at this IOBC meeting is of great importance to us.

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

Julie Smith<sup>1</sup>, Clare Tucker<sup>2</sup>, Charlotte White<sup>3</sup> & Pete Berry<sup>4</sup>

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

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

Eight field experiments were conducted across three cropping seasons in the UK (2012 – 2014). The objective was to quantify the effects of fungicide on yield, mediated through means other than the control of visible disease. The boscalid-picoxystrobin co-formulation was applied at mid flowering (BBCH 65) and evaluated against boscalid, prothioconazole, boscalid-metconazole co-formulation and an untreated control. Disease incidence and sever-

ity was assessed and physiological measurements included photosynthetic rate, transpiration efficiency, water use efficiency measured at the canopy and leaf level, leaf retention, leaf greenness, leaf and pod green area indices (integrated through time to give 'healthy area duration' [HAD] values), intercepted light, canopy senescence, lodging and yield.

Sclerotinia stem rot and light leaf spot incidence ranged from 0–50% across the experiments. Cross-site analysis showed that yield was significantly higher in boscalid-picoxystrobin treatments by a mean of 0.23 t ha<sup>-1</sup> after disease effects had been accounted for. This was achieved through increases in both seed size and seed number. Effects of boscalid-picoxystrobin treatment on the canopy were significant. HAD was increased as a result of increases in green canopy area (largely through better green leaf retention) and canopy duration (due to delayed senescence). A strong positive association was found between yield and HAD with each unit of HAD increasing yield by approximately 0.06 t ha<sup>-1</sup>. Consistent trends were observed for increased leaf and pod greenness, indicating a higher chlorophyll content following fungicide application.

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

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

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

Cezary Tkaczuk<sup>1</sup>, Anna Majchrowska-Safaryan<sup>1</sup>, Witold Irzykowski<sup>2</sup> & Małgorzata Jędryczka<sup>2</sup>

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

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

persistence and compatibility with pesticides, predators and parasitoids should be investigated.

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

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

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

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**Abstract:** Pollen beetles are a main pest in oilseed rape throughout Europe. A laboratory screening of Swiss isolates of entomopathogenic fungi revealed high potential of *Beauveria bassiana* for biological pollen beetle control. To improve efficacy of fungus spore suspensions in the field, we explored formulations with vegetable oils or stone dusts that have previously been shown to reduce pollen beetle abundance in the field. The combined application of fungus spores and vegetable oil

showed increased pollen beetle mortality in laboratory experiments and indicated increased yield in a field trial. To reduce fungus spore inactivation by ultraviolet irradiance upon field application, natural compounds were tested as UV-protectant additives to spore formulations. Formulation of *B. bassiana* spores with natural UV protectants tested in laboratory bioassays increased the number of surviving spores up to a factor of two, relative to untreated spores.

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



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

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

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

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

1. WOSR with applications of Metazachlore
2. WOSR with applications of a suite of alternative herbicides to Metazachlore
3. WOSR with under sowing (*Colza associée*)
4. WOSR in extended row space (75 cm) with herbicide applications only within rows

5. False-flax (*Camilina sativa*)
6. Flax (*Linum usitatissimum*)
7. WOSR with mechanical weeding
8. WOSR in biological cultivation without chemical control measures

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

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

The monitoring of the weed communities was done at all three sites in autumn and spring (after weed control measures). Results of four out of the eight experimental treatments (Metazachlore, herbicide alternatives, extended row space and biological cultivation) are presented here. Overall up to 25 different weed species were identified. Experimental sites showed significant differences concerning the species distribution as well as in the number of species and total number of weeds per square meter. Reisdorf showed the lowest total number of weeds followed by Hobscheid and Flatzbour. Chickweed (*Stellaria media*) and

Knotweed (*Polygonum* spp.) were mainly found in autumn. A tendency towards brassicaceous weed species was not found except for Shepherd's purse (*Capsella bursa-pastoris*) that was mainly found in springtime at Flatzbour. Species that were mainly observed in autumn disappeared due to the chemical and mechanical control measures and were replaced by other weed species in the following spring e.g. Field pansy (*Viola arvensis*), Common poppy (*Papaver rhoeas*) and Poisson hemlock (*Conium maculatum*). Both herbicides Metazachlore and the alternative herbicides did not perform well at Hobscheid due to the sandy soil

**Key words:** herbicides, cropping system, weed communities

characteristics. Results are still preliminary and will be validated in the next four years of the project.

**Acknowledgements** We gratefully acknowledge the financial support of the Ministère de l'Agriculture, de la Viticulture et de la Protection des Consommateurs of Luxembourg, Ministère du Développement durable et des Infrastructures and the Ministère de l'Enseignement supérieur et de la Recherche. Additionally, we thank the farms for technical support at the experimental sites and Gilles Parisot (Chambre d'Agriculture) for his fundamental support in weed identification.

**Insecticides:  
need, efficacy  
and side effects**

## Arthropod pest control for UK oilseed rape – expert opinions on insecticide efficacies, side effects and alternatives

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

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**Abstract:** Large scale field trials were performed in spring 2013, 2014 and 2015 to assess the effects of Pymetrozine (2013–2015), Tau-fluvalinate (2013–2015), Thiacloprid (2013–2015), Chlorpyrifos-ethyl (2013–2015), Phosmet (2013–2014) and Indoxacarb (2015), used to control the pollen beetle *Meligethes aeneus* (F.) (Col.; Nitidulidae), on pollen beetle populations and their parasitic wasps in winter oilseed rape. The insecticides were applied at the recommended rate for commercial use soon before flowering on large strips of oilseed rape (30m x 200m), divided into four plots of 50m x 30m. A strip was left untreated as control. Insects were sampled weekly using beating methods and sweep netting from day 1 to 50 days following product application. The direct effects of the products were assessed on adult pollen beetles (target pest) and adults of parasitic hymenoptera. The long term effects were assessed on pollen beetle larvae to determine their number, the parasitism rate and to estimate the balance of parasitic hymenoptera/pollen beetle that could be produced by each product scenario for the next season.

According to the results obtained, the insecticides

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

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

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

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**Abstract:** Pyrethroid resistant pollen beetles (*Meligethes aeneus*) are now widely distributed in Germany since the first resistant beetles were detected about 15 years ago. Over half (53 %) of sensitive populations in biotests in 2005 had declined to 0 % by 2011. Very clear resistance increased from 33 % in 2005 to 100 % in 2015. Monitoring of the other insect pests of oilseed rape in Germany

showed no resistance yet in pest species including *Ceutorhynchus napi* and *C. pallidactylus*, *Dasineura* spp. and *Phyllotreta* spp., but resistance to pyrethroids has recently developed in *C. obstrictus* and *Psylliodes chrysocephala*. Resistance of both species has spread over many parts of Germany but resistance factors in laboratory biotests are far below resistance values known for *M. aeneus*.

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

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

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**Abstract:** The cabbage stem flea beetle and rape winter stem weevil are two major pests of winter oilseed rape (WOSR) in France. Since 2009, certain areas of France have suffered from increased pest infestations despite repeated treatments to control them. The phenomenon has increased year after year. Terres Inovia and its partners, through the AFPP (French Association for Plant Protection) "Insect resistance" group, have confirmed that several populations of these two pests are resistant to pyrethroids and that multiple mechanisms are involved. A mutation in the sodium channel gene known to be linked to the knock-down resistance (kdr) phenotype was discovered in the cabbage stem flea beetle. The mutation is widely spread in French territories where resistance is found. A second mutation known as Super-knock down resist-

ance (skdr) was also detected in the pest. This mutation is found in the East of France, mainly in the Yonne region, but its distribution seems to be bigger. For rape winter stem weevil, the kdr mutation was found but not skdr. Furthermore, we showed with inhibition tests that resistance by detoxification is combined with these mutations to confer a high level of resistance to lambda cyalothrin in these two pest species. A complex combination of kdr, skdr and metabolic based resistance seem to confer different levels of resistance in the field.

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

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

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

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**Abstract:** Populations of Czech *Meligethes* were simultaneously tested for susceptibility to lambda-cyhalothrin and chlorpyrifos-ethyl and also to lambda-cyhalothrin and thiacloprid in 2014. In 2015, *Meligethes* populations were again tested for susceptibility to lambda-cyhalothrin and chlorpyrifos-ethyl and to lambda-cyhalothrin and thiacloprid simultaneously, this time using populations of mainly Czech but also Slovak origin. IRAC adult vial tests were used. IRAC no. 011 v. 3 for lambda-cyhalothrin, IRAC no. 025 for chlorpyrifos-ethyl and IRAC no. 021 for thiacloprid. For each of the tested populations the LC<sub>50</sub> and LC<sub>90</sub> values for the three insecticides were

determined. Correlation analyses were made with transformed ( $\log_{10}$ ) LC values. Significantly negative correlations were recorded for the LC<sub>90</sub> and LC<sub>95</sub> values estimated for the correlation between lambda-cyhalothrin and chlorpyrifos-ethyl. Pyrethroid resistance in pollen beetle populations should indicate their slightly higher susceptibility to chlorpyrifos-ethyl. Contrary to expectations, significant positive correlations were recorded for the LC<sub>50</sub>LC<sub>90</sub> and LC<sub>95</sub> values for the correlation between lambda-cyhalothrin and thiacloprid in both years. Pyrethroid resistance in pollen beetle populations should indicate their lower susceptibility to thiacloprid.

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



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

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

In 2013–2015, field trials were conducted on crops of winter oilseed rape in the region of Braunschweig (northern Germany). Insecticide treated and untreated plots of approximately 1000 m<sup>2</sup> were established in a randomized block design with four replications. Overwintered pollen beetles were counted before, and several days after application of Biscaya. Plant samples were collected 1–2, 7–9 and 14 days after application. Buds (> 2 mm) of the main stem and two side shoots were dissected to assess the number of eggs and larvae.

New generation beetles were collected using soil-photoelectors. In addition, greenhouse trials were done using untreated and insecticide-treated pollen beetles and plants collected from untreated and insecticide treated plots, respectively. Ten pollen beetles were caged on individual inflorescences using perforated plastic bags (10 replicates each). After 3–4 days the vitality of beetles was assessed and the buds dissected for eggs and larvae.

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

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

## First data on RNAi to control pollen beetles

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**Abstract:** The pollen beetle, *Meligethes aeneus*, is one of the most economically important pests in European agriculture and is one of the most destructive pests of cruciferous oilseed crops. The resistance to pyrethroid insecticides in recent years in several EPPO countries has led to an increased need for novel pest control strategies such as RNA interfering (RNAi) for controlling the insect. The aim of this work is to evaluate the RNAi technique for

investigating the functional genomics and its potential for controlling *M. aeneus*. The transcriptome data were deployed in order to find the candidate essential genes and subsequently, to design double-stranded RNA (dsRNA). To address the functionality of these essential genes, dsRNA was delivered to larvae by means of microinjection. Based on our preliminary data, the RNAi technique can be used as a novel method for controlling this important pest.

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

# Pyrethroid sensitivity of adults and larvae of *Meligethes aeneus*

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

Overwintered pollen beetles were collected in 2015 and 2016 at different locations in Romania, Hungary and northern Germany. The pyrethroid sensitivity of random samples of beetles was tested using the Adult-Vial-Test (AVT, IRAC Method 011) but with an assessment after 5 instead of 24 hours. Lambda-cyhalothrin (e.g. in Karate Zeon) was used. Different rates from 0.003 to 0.375  $\mu\text{g}$  lambda-cyhalothrin  $\text{cm}^{-2}$  were used with four replications each. To obtain pollen beetle larvae, oilseed rape was cultivated in the greenhouse at

15°C and 15 beetles from the same batch were caged per plant and allowed to oviposit for 12–13 days. The species of caged adults was determined at the end of this period. The plants were kept at 15°C in large saucers and the pots were covered with plastic bags to prevent larvae burrowing into the soil. Between 7–8 days after the egg laying period L2-larvae were ready to pupate and were collected in the saucers. Plants were fogged with water to enhance emigration of larvae. Larvae were inserted into coated glass vials similar to adults in the AVT. On the lid of the vials 2  $\mu\text{l}$  of water was added to increase humidity. Vitality of larvae was assessed 5 hours after insertion. LD<sub>50</sub>-values were calculated using POLO PLUS 2.0.

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

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

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

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**Abstract:** We conducted two years field experiments in Germany comparing conventional and dropleg spraying techniques. We found slightly reduced efficacy of dropleg spraying of Biscaya and Mavrik on oilseed rape at BBCH growth stage 65 (full flowering) against insect pests (*Meligethes aeneus*, *Ceutorhynchus pallidactylus*, *C. obstrictus*

and *Dasineura brassicae*). The dropleg technique therefore seems suitable for control of insect pests during the flowering stage of oilseed rape, when exposure for bees and other pollinators needs to be minimized. When testing Biscaya and Mavrik in 2015, the latter had lower efficiency.

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

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

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

OSR is the third most widely grown crop in the UK, after winter wheat and barley, covering 653,000 hectares in 2015 (DEFRA, 2015) and with an average yearly value of £804M (Nicholls 2016). It is an important break crop in a cereal rotation and provides an important temporal spike of floral resources for flower visiting insects. Since the restriction came into force, farmers in the south/east of England have been reporting severe crop damage primarily from attack by the cabbage stem flea beetle (*Psylliodes chrysocephala*), which affects around 67% of the OSR grown in the UK, and accounting for a loss of 3% of the national production (Wynn *et al.*, 2014). There is also growing concern of a potential impact from turnip yellow virus (TuYV), transmitted by the peach-potato aphid (*Myzus persicae*) with 72% of winged females carrying the virus (Stevens *et al.*, 2008) which can cause yield losses up to 26% in the UK, pre restriction (Stevens *et al.*, 2008). Little is known about how TuYV transmission will be impacted by the neonicotinoid restrictions. Both *P. chrysocephala* and *M. persicae* were controlled by the use of neonicotinoid seed dressings and subsequent pyrethroid foliar sprays in the autumn. Since

the restriction it has been reported that the control threshold levels of five *P. chrysocephala* larvae per plant, has been exceeded in 46% of the planted crop (White, 2016). This has led to pyrethroid sprays being applied up to four times per crop in an attempt to control the beetles (White, 2016). However, both *P. chrysocephala* and *M. persicae* are known to be resistant to pyrethroids. *Psylliodes chrysocephala* is known to exhibit resistance in continental Europe (Hujland *et al.*, 2015) and in September 2014 resistance was recorded in the UK and has been shown to be present in 73% of the adults tested. The peach-potato aphid has also been known to be resistant to pyrethroids for a number of years (Devonshire & Moores, 1982) and may be capable of developing resistance to neonicotinoids (Bass *et al.*, 2011). Thus, the use of pyrethroids may be ineffective at controlling the pests, even increasing resistant populations, but could well be detrimental to the beneficial insect population. Given the restriction on neonicotinoid use, coupled with pyrethroid resistance and projections of milder winters in the UK, which will increase the risk of pest damage, the impact of both *P. chrysocephala* and TuYV on OSR production in the UK is expected to increase. It has already been suggested that 38,000 ha extra OSR would have been planted if seed dressings were still available (White, 2016) highlighting the farmers response to the restriction.

To better understand the full implications of the neonicotinoid restriction, it is important to examine how alternative methods of pest control impact crop protection, non-target insects including pollinators and productivity. Field trials at Rothamsted Research will test a variety of past, present and future chemical, and non-chemical Integrated Pest Management (IPM) practices in winter OSR. These will include neonicotinoid seed dressings, pyrethroid sprays, trap cropping, nurse cropping, elevated sowing rates and winter cutting. Pest abundance and damage, natural enemy

communities, flower production, pollinator communities and yield will be assessed. These data sets will allow the interactions of pest management practice, yield and impact on and from beneficial insects to be quantified in winter OSR. The ultimate goal of this project is to run a cost:benefit analysis

on multiple IPM tactics in winter OSR to incorporate pest management, ecosystem service provision and crop productivity. This will not only highlight the impacts of different pest management approaches on crop yield but also explore the implications for ecosystem service provision and farmer profits.

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

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**Abstract:** Winter oilseed rape (*Brassica napus* L.) is a host plant for numerous pest insects. Pests are preferentially controlled by agronomic methods. However, when those methods are insufficient to prevent a surpassing of the economic damage threshold, insecticides are applied. Because of potential side-effects on beneficial organisms like honey bees (*Apis mellifera* L.), exposure of pollinators to insecticides should of possible be prevented by the method of application. It is a matter of dispute whether or not the prevention of exposure, particularly of pollinators, to neonicotinoid insecticides is sufficiently effective under field conditions. Here, we tested if the area covered by oilseed rape, the distance between the nearest oilseed rape crop and the size of the biggest oilseed rape crop in radii of 2 and 5 km around >150 apiaries

in Luxembourg were correlated (Pearson,  $P < 0.05$ ) with winter honey bee colony losses (% per apiary). In the period 2010–2012, when neonicotinoid use was permitted and widespread as both spray application or as seed coating, no significant correlations were detected between oilseed rape land use parameters and honey bee colony losses in the winter following oilseed rape harvest – except for 2012 in northern Luxembourg, where the size of the biggest oilseed rape crop was positively related with honey bee colony losses within the 5 km radius around the apiaries. This individual observation was, however, neither reproducible in space, nor in time, so far. Our study thus provided no reproducible evidence for an effect of winter oilseed rape on honey bee colony winter losses in a region and period where neonicotinoid use was widespread.

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

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## Improving decision support for IPM



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

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**Abstract:** A better understanding of pest biology can lead to improved methods for integrated pest control. Until now there is limited knowledge on pest population multiplication, though insect pest numbers attacking crops in the following season are important for future pest pressure predictions and improved IPM. The number of next generation beetles in winter oilseed rape can be collected using ground photoelectors. This method was used in 44 fields in Germany 2015 and nearly 100 % of the next generation beetles of *Meligethes aeneus*, *Psylliodes chrysocephala*, *Ceutorhynchus obstrictus*, *C. pallidactylus* and *C. picitarsis* hatched from the soil within the eclectors were caught if the eclectors were kept in the field from BBCH 75–78 until harvest. Average / max. number of beetles caught per m<sup>2</sup> were: *M. aeneus* 212 / 3000, *P. chrysocephala* 298 / 2000, *C. obstrictus* 88 / 637, for *C. pallidactylus* 25 / 152, and for *C. picitarsis* 7 / 12 (only detected in South Germany at 3 locations). For *M. aeneus* the pest pressure in 2016 was limited in accordance with the low number of new generation beetles found in 2015. Population density of most species was quite evenly distributed within Germany but for *C. obstrictus* high densities occurred throughout the north and east of Germany, finally resulting in high pressure in this region in May 2016. Such monitoring data could give hints for areas with high pest attack in the next season, although winter or aestivation mortality still needs consideration. The data can also address questions on sustainable effects of insecticides and will lead to a better understanding of pest biology.



**Figure 1.** Ground photoelector. The base ring (0.25 m<sup>2</sup>) is dug into the soil just after winter without disturbing the crop plants within and outside the ring and the tent is fitted at about BBCH 75–78, leaving all plant material grown inside the ring by bending it down before fitting the tent. The eclectors were then left until harvest date. At the top a trapping box or, for live insect catch, a perforated plastic bag, is fixed to catch all insects escaping through the opening supplying light. For pests such as *Dasyneura brassicae* and *C. napi* such traps would have to be established in the crops following oilseed rape.

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## Climatic factors help predict stem weevil abundance

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**Abstract:** Biological and ecological features of the rape stem weevil (*Ceutorhynchus napi*) and cabbage stem weevil (*Ceutorhynchus pallidactylus*) are similar and they are often presented as a complex, although life cycles have important differences and demand different approach in their control. The critical point to determine the optimal time for insecticide treatment is the time of their migration into winter oilseed rape crops. Based on climatic factors various phenological forecasting systems have been developed and help producers to predict the date of pest immigration into crops. During four growing seasons, adult forms of *C. napi* and *C. pallidactylus* were trapped using yellow water traps at six locations within five Croatian counties with intensive oilseed rape production. The content of

each trap was emptied once a week. Climatic data were obtained from the Croatian Meteorological and Hydrological Service for each year of the investigation and for each location. Exploratory data analysis, using regression tree analyses was done in R 2.30, applying the package “tree”. Factors that can help in prediction of appearance and abundance of *C. napi* are: the growth stage of oilseed rape plants, daily sunshine hours and mean daily air temperature; for prediction of *C. pallidactylus* an additional important climatic factor was mean daily precipitation. Climatic factors are very important for migration of stem mining weevils from their hibernation places to oilseed rape fields and together with the growth stage of the crop are very useful in predicting the appearance of adult forms.

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

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

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**Abstract:** Winter oilseed rape (*Brassica napus* L.), (WOSR), is an important host plant for numerous pests throughout the entire growing season. The cabbage stem weevil, *Ceutorhynchus pallidactylus* (Mrsh.) (Col.: Curculionidae), is commonly found in WOSR throughout Europe. The larvae of this species mine within the petioles of plants in spring time, resulting in yield losses up to 20%. As well as knowledge about the migration activity of this pest, information on the population size can be helpful in decision support; helping farmers to optimize pesticide applications. Eickermann *et al.* (2015) published a model based on long-term multi-site datasets from field observations using yellow water traps. The monitoring was conducted from 2007–2012 on 5 locations in Luxembourg. The developed model estimates if the economic threshold of *C. pallidactylus* (> 10 individuals per trap within 3 consecutive days) will be breached in March, in relation to the mean winter air temperature

in different periods in previous February. If mean winter temperatures between 5 and 13 February are closer to 4.0 °C than to –2.4 °C, no breach of the control threshold is to be expected in the subsequent March. The model allowed a forecast with an accuracy of 81.5% and was validated in the years 2013 to 2016 for the area of Luxembourg.

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

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

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

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

Over-use of cheap and effective insecticides has led to heavy selection pressure for resistance and pyrethroid-resistant pests are now widespread across Europe (e.g. Thieme *et al.* (2010); and papers in this volume: Heimbach & Brandes (2016); Robert *et al.* (2016), Seidenglanz *et al.* (2016); Brandes & Heimbach (2016)). To reduce further selection pressure and to aid optimal and judicious use of the more expensive active ingredients, on-line decision support system (DSS) tools driven by local

meteorological data can help to focus monitoring effort to when it is most needed. We assessed the proPlant.expert DSS (Newe *et al.*, 2003) for pollen beetles (*Meligethes aeneus*) in the UK and found that using it could almost halve monitoring effort compared with following conventional monitoring advice (Ferguson *et al.*, 2015). We describe how a free version of this, the Bayer Pollen Beetle Predictor, available to growers in the UK can be used to reduce monitoring effort, save unnecessary prophylactic sprays and help time spray application.

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

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

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

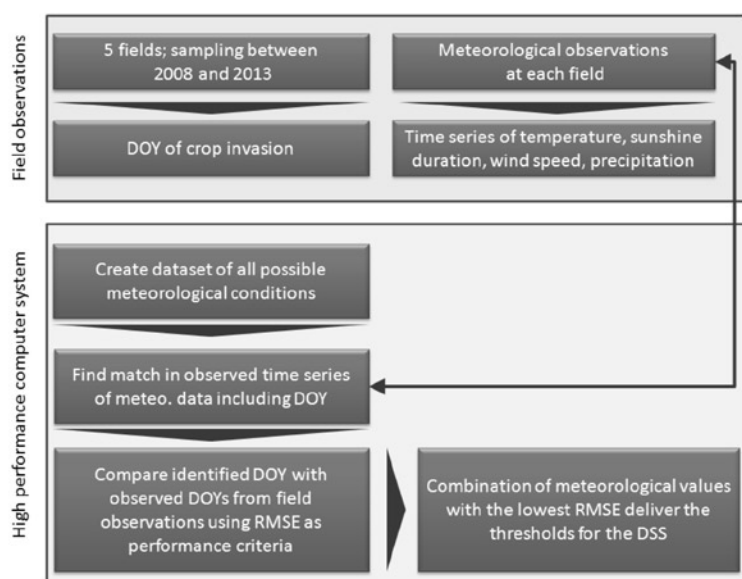
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**Introduction** The pollen beetle, *Meligethes aeneus* (Fabricius), is a common pest insect in winter oilseed rape (WOSR) in Europe and responsible for possible yield losses of up to 80% (Hansen, 2003). The extent of field losses depends on meteorological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

sion by *M. aeneus* in spring. The setup of those DSS is time consuming and requires high computational resources. Therefore, a high performance computer is a suitable tool for the development of such DSS.

**Materials and methods** The dataset to develop the phenological model consists of field observations of the date of first activity of *M. aeneus* together with local meteorological measurements. During the period from 2008 until 2013, data were obtained from five field sites in Luxembourg: Burmerange, Christnach, Obercorn, Reuler and Useldange. At each site, immigrating individuals of *M. aeneus* were caught with yellow water traps (four per site, three times per week between February and June) based on Williams (2010). Meteorological data were obtained from automatic weather stations located directly beside each of the five test fields. Daily values of air temperature, mean soil temperature (0.05 m depth), precipitation and sunshine duration were retrieved and pre-processed via an automatic data



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

processing chain for gap detection, quality and plausibility checking. A threshold-based model for predicting migration of *M. aeneus* into WOSR was developed. To optimize computational resources, plausible ranges of the meteorological variables were defined for the later analysis: mean air temperature 0–30 °C; mean soil temperature 0–20 °C; sunshine duration 0.1–12 h. The method for determination of the final threshold values is shown in Figure 1. All possible combinations of air temperature, soil temperature and sunshine duration, with increments of 0.1 were compared to the time series of the observed meteorological data at the respective field. Combinations that did not exist in the measured data sets were excluded from further analysis. The day of year (DoY) when the combination of meteorological variables first occurred in the measured time-series was identified and compared with the date of first crop invasion using the root mean squared error (RMSE) as quality criterion. Precipitation was included because it hinders flight activity and threshold values of 0.2,

0.5, 1.0, 2.0, 3.5, or 5.0 mm (daily totals) were tested. In addition, the persistence of two and three consecutive days of all threshold values was assessed.

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

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

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

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

In autumn 2015 YWT in 5 variants (AG: angular with grid; A: angular without grid; RG: round with grid; R: round without grid; RD: round dug into the ground without grid) with at least 2 replicates were placed in 17 different winter oilseed rape fields all over Germany. The traps were filled with a 10% benzoic acid water solution with de-

tergent with about 5 m distance between each trap on 1 side of a field. The grids used had a mesh size of 7 mm and were supplied together with the traps. Rectangular traps were sponsored by Syngenta and round traps by BayerCropscience.

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

**Table 1.** Mean percentage of total catch in different yellow water traps (+ SD) at 17 field sites with at least 150 beetles per site (58 replicates altogether); n = 16 332 beetles captured in September and October, 2015. (AG: angular with grid; A: angular without grid; RG: round with grid; R: round without grid; RD: round dug into the ground without grid).

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

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

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

**Key words:** yellow water trap, cabbage stem flea beetle, bees

**Acknowledgement** The work was carried out in cooperation with German plant protection services and supported by the Union for Promoting Oil and Protein Plants e. V. (UFOP).

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

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**Abstract:** The within-field spatio-temporal distributions and relationships of *Ceutorhynchus pallidactylus* (Marsham) (Coleoptera: Curculionidae) and *Meligethes aeneus* (Fabricius) (Coleoptera: Nitidulidae) in a crop of winter oilseed rape [*Brassica napus* L. (Brassicaceae)] were assessed in the Olomouc region (northern Moravia, Czech Republic) over the course of 2013–2015. Distributions were analysed using SADIE analysis (Spatial Analysis by Distance IndicEs), for associations we used Quick association analysis. In 2013, males and females of *C. pallidactylus* were within-field associated during the period of higher flight activity when beetles were rather edge distributed. The similar character of distribution patterns and significant association of both

sexes of *C. pallidactylus* was recorded in mid-April 2015. Despite a random distribution of females of *C. pallidactylus* in the crop on April 22 2013 there was a significant spatial association between the adults of *M. aeneus* and the females that date. Significant association between non-randomly distributed females of *C. pallidactylus* and adults of *M. aeneus* was recorded on 21 April in 2015. In all years, adults of *M. aeneus* showed stronger tendency to aggregation in crops mostly during their higher flight activity. When populations of the assessed insects were non-randomly distributed in crops ( $I_a > 1$  for  $p < 0.05$ ), they were usually aggregated into one major and one adjacent patch clusters localized along the edge of field with tendencies to spread to central parts of crop, too.

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



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

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**Abstract:** An easy but not widely used method for estimating the number of *P. chrysocephala* larvae was tested in this study. The Funnel-Method can be considered as a modified Berlese-Funnel-Method with no heat supply. The passive extraction of cabbage stem flea larvae by using a funnel and a catching vessel generated nearly the same results as the manual dissection. The big advantage of using the Funnel-Method is that distinctly less

labour time is needed; the disadvantage is that the extraction needs about 21 days until all larvae have left the plant tissue and that larval development stages cannot be related to the collection date. The results of this study indicate that the Funnel-Method is a cheap and simple tool for practical use of estimating the total number of larvae per plant, but with a time delay before results are available.

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

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

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

Current control methods rely heavily on the use of insecticides, but overuse and poor timing has led to the development of insecticide resistant popula-

tions of pollen beetles throughout Europe (reviewed in Thieme *et al.*, 2010). Decision-support tools (such as proPlant) can be used in order to optimise use and reduce monitoring effort (Johnen *et al.*, 2010; Ferguson *et al.*, 2015). This tool is based local meteorological conditions and predicts migration events up to three days in advance. However, there is potential to use long-term spatially-explicit data to elucidate trends over time that could reveal other ecological mechanisms and thus improve predictions.

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

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## **Delivering effective bio-control of OSR pests via natural enemies**

## Valuing natural pest control services for UK arable crops

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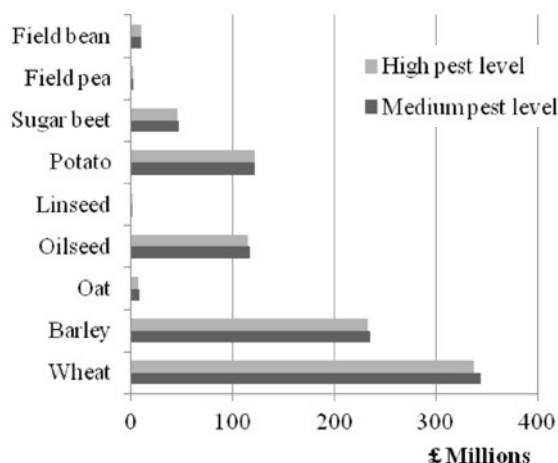
**Introduction** Agriculture is a complex system that relies on various ecosystem services (Zhang *et al.*, 2007). Natural pest control is an important regulating service, which can help to suppress pest damage and maintain crop yield (Power, 2010). However, the importance of natural pest control is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

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

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

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

Under the low pest damage level scenario, where arthropod pests would not cause damage to the crop yields even without natural enemies in the



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

field, no values would be generated from natural pest control (Figure 1). Under the medium damage scenario, where pests would exceed the economic damage thresholds without natural enemies, both conventional and IPM systems would benefit from natural enemies by the increased crop yields, and IPMs also the reduced insecticide costs. For high pest densities, where natural enemies would not be able to suppress pests below the economic

threshold, IPM users would not gain from the insecticide cost reductions, but increased crop yields would still apply for both systems. According to experts' opinions, natural enemies could also provide pest control services to conventional farmers. Our study provides insights into valuing natural pest control services for UK arable crops. Limitations exist and further studies are needed for evaluations.

**Acknowledgements** We sincerely thank the participants from the Association of Independent Crop Consultants who provided valuable information for this study.

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## Nitrogen fertilization alters host selection of pollen beetle parasitoids

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

Oilseed rape plants were fertilized with four

different N levels: 0, 80, 100, 160 kg N ha<sup>-1</sup>. Out of 957 *B. aeneus* larvae collected in total, 90.7% were uninfested by parasitoids. In the remaining larvae, five parasitoid species were found to be responsible. Of parasitised larvae, 76.4% were infected once and the rest had two or more parasitoids. Although the number of pollen beetle larvae was much higher in N160, parasitoid rate was greatest in N80. This may be related to N-dependent production of VOCs, which helps insects in host detection. These results suggest that the ecological control of pollen beetle herbivory is most promising under moderately high N addition rates (treatments 80 and 100) compared with non-fertilized or strongly fertilized treatments.

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

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

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**Abstract:** The pollen beetle, *Meligethes aeneus*, is one of the most important pests of oilseed rape (OSR). It is known to be strongly influenced by the proximity of woodlands and grassland. Moreover, studies on the attractiveness of other species in the Brassicaceae suggested that trap crops near woodlands could help to contain their spread. Another option would be to preserve its main parasitoid, *Tersilochus heterocerus*, which can induce very high parasitism rates. Here we investigated jointly the impact of proximity to grassland and woodlands on the abundance of adults of both species. We first show there is no pollen beetle gradient within fields but a

gradient up to 6,000 m even when intermediary OSR crops are present, suggesting trap crops would not efficiently halt pollen beetle. Multivariate regression confirms the strong positive impact on pollen beetle abundance of woodland density within 200 m and of grassland areas within 400 m. Only the presence of grassland significantly impacts the presence of parasitoids. This suggests that pollen beetle management could be improved by adequate temporal management of grasslands. For example, farmers could support grassland production of nectar-producing flowers during parasitoids' spring flight but mow them before the summer flight of the pollen beetle.

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

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

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**Abstract:** The cabbage seed weevil, *Ceutorhynchus obstrictus*, is an important oilseed rape crop pest in Europe. Its abundance is usually managed by synthetic insecticides that can be harmful to neutral and beneficial organisms, including parasitoids, occurring in the agricultural fields. Parasitoids can play an important role in the control of the population size of seed weevils.

This experiment was conducted to see if and how different field bordering element types affect cabbage seed weevil infestation and parasitism rate in conventionally grown winter oilseed rape crops. The percentage of damaged pods was low (between 8.5% and 10.9%), but even with such low pest abundance the parasitism rate was sufficient for efficient biocontrol; varying between 55.5% and 68%.

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



**Breeding for resistance  
to insect pests for IPM  
strategies in OSR**

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

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

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

## Potential for oilseed rape resistance in pollen beetle control

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**Abstract:** The pollen beetle (*Meligethes aeneus* F.) is one of the most damaging pests of oilseed rape (*Brassica napus* L.). A number of strategies are currently developed to combat this insect species, including synthetic and botanical insecticides, trap cropping and biological control by the pest's natural enemies. One strategy, plant resistance, although widely used in the manage-

ment of many crop species, has not yet been developed against the pollen beetle despite a few notable attempts. Here we summarize the work of our laboratory on (i) the drawbacks to applying this strategy and how to circumvent them, and (ii) how the natural resistance present in oilseed rape may be used to limit pollen beetle damage in a theoretically effective and sustainable manner.

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

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

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**Abstract:** Pollen beetle (*Meligethes aeneus*) is one of the main insect pests affecting oilseed rape crops. Efficiency of insecticide use to control this pest is decreasing due to its adaptation to phytosanitary products such as pyrethroids. In this context, alternatives to this kind of control need to be found. Breeding oilseed rape for resistance to insect attacks could be an interesting way to deal with this issue. Actually, the primary benefit of this approach is the ease of its use by farmers. However, it remains complicated to breed plants for insect resistance, especially using field tests on large genotype collections. Our knowledge of the chemical ecology of interactions between oilseed rape and pollen

beetles could allow us to find biochemical markers of this resistance. In this way an indirect breeding approach based on markers of resistance could be adopted rather than direct confrontation of plants to insects. Laboratory tests have already shown that variation in resistance between oilseed genotypes could be explained by the biochemistry of bud tissues. These observations now need to be validated under field conditions. To test this, we conducted a multi-site experiment in France to observe resistance of different genotypes to pollen beetles. Over three locations we phenotyped pollen beetle damage and sampled buds in the field to analyse their chemistry. Here we present the results of this experiment.

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

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

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

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

The same set of genotypes was also tested in no-choice and dual-choice feeding experiments un-

der controlled laboratory conditions. Single excised buds 2–3 mm in size were offered to one field-collected overwintered PB in small transparent plastic boxes (30 replicates per genotype). The amount of bud tissue consumed within 24 h was measured by the loss of biomass, based on the fresh weight of buds at the start and the end of the experiment.

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

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

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

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

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**Abstract:** The cabbage seed weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) is an important pest of brassicaceous oilseed crops in Europe and North America. Currently, application of chemical insecticides is the only commercially used strategy to control adult weevils and reduce subsequent yield losses associated with larval feeding on seeds. In previous studies, *Sinapis alba* (L.) has been found to be resistant to *C. obstrictus*, although the mechanisms of resistance still remain unknown. In a two-year study, we determined the

relative susceptibility of different introgression lines (*S. alba* x *B. napus*) to weevil attack under controlled conditions and in semi-field trials. Specifically, we assessed the oviposition preferences of adult females and different parameters of larval performance. Our results indicate that antixenosis and antibiosis are expressed by individual introgression lines, with stronger effects of antibiotic mechanisms of resistance observed in both years. In addition, the resistance of *S. alba* to *C. obstrictus* was confirmed in no-choice tests as well as in semi-field trials.

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

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

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

ness to oviposition by adult *D. radicum* females.

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

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

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

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## Resistance to blackleg

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

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**Abstract:** Blackleg disease, caused by *Leptosphaeria maculans* is one of the most important fungal diseases in oilseed rape (OSR) production world-wide (Fitt *et al.* 2006). Genetic resistance is an important tool to control this disease. Seedling resistance is conferred by single major genes. Due to its sexual propagation, *L. maculans* isolates are capable of evolving rapidly from avirulent to virulent strains on cultivars harbouring major resistance genes. Therefore, resistance of oilseed rape against *L. maculans* conferred only by major resistance genes was often overcome and led to severe yield losses in the past (Rouxel *et al.* 2003; Sprague *et al.* 2006). The aim of this study was to determine the efficiency of major resistance genes to *L. maculans* in different oilseed rape growing regions in Germany by identifying the frequency of virulent isolates and determining the race spectra of *L. maculans*. We cultivated two oilseed rape cultivars in fields throughout Germany from 2011 to 2014. 'NK Bravour' harbouring for the most part no known major genes against *L. maculans* (serving as trap crop) and 'Exocet' harbouring the major gene *Rlm7* to

observe resistance breakage in the field. In autumn and spring we collected true leaves with typical Phoma lesions to gain isolates of *L. maculans*. Single pycnidia isolates were tested with an oilseed rape differential set through cotyledon inoculation for their virulence to different major genes. The differential set consisted of 10 oilseed rape genotypes harbouring the major genes *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm7*, *Rlm9* and *LepR1*, *LepR2* and *LepR3*. Thereby, the frequency of virulent isolates in a region was determined. Isolates showing the same virulence complement were grouped to the same race. The frequency of isolates being virulent to *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4* and *Rlm9*, respectively, was above 85%. Conversely, the frequency of virulent isolates to *Rlm7* was very low (< 5%). Interestingly, the frequencies of isolates being virulent to the major genes *LepR2* and *LepR3* showed a considerable variability between different regions, ranging from 35% to 100%. There was no isolate showing virulence to *LepR1*. Most isolates belonged to two races with a high virulence complexity. Only *Rlm7* and *LepR1* are still mediating resistance in oilseed rape to German *L. maculans* populations.

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



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

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**Abstract:** Stem canker of brassicas (blackleg), caused by the fungal complex *Leptosphaeria maculans*–*L. biglobosa* is a damaging disease of oilseed rape (*Brassica napus*) worldwide. Nowadays, the incorporation of *L. maculans* resistance into *Brassica* lines with desirable agronomic and quality traits is a major concern for breeding programs. Every year new population and hybrid varieties are introduced to the market. The aim of this study was to identify the sources of genetic resistance to stem canker in *Brassica* hybrids obtained from the crossings between two high yielding cultivars of *B. napus* and *B. carinata*, *B. fruticulosa*, *B. rapa* ssp. *chinensis* and *B. rapa* ssp. *pekinensis* as well as to check the resistance and performance of winter oilseed rape cultivars under field conditions in the western region of Poland. The experiment was conducted in field conditions using the F<sub>2</sub> generation of these interspecific hybrids. Moreover, 44 cultivars of winter oilseed rape, officially registered in Poland by the Research Centre for Cultivar Testing (COBORU) and two candidate cultivars with *Rlm7* resistance gene were

tested. Screening of plant susceptibility/resistance was done in 2015. The field experiment was done in a randomized complete block design with two replicates in two locations in the Wielkopolska region (Greater Poland), situated 80 km apart. Disease incidence was assessed in autumn, two months after sowing, on 50 plants per replicate, according to a 0–4 scale of disease severity. Furthermore, the determination of *Leptosphaeria* species was studied using Loop-mediated DNA Amplification (LAMP) method. The genotypes differed with their reaction to the pathogen. Forms with increased resistance to blackleg especially among *B. napus* × *B. carinata* and *B. napus* × *B. fruticulosa* hybrids have been found. In both locations, the cultivars bearing the *Rlm7* resistance gene showed significantly less symptoms of stem canker, as compared to cultivars with no *Rlm7*. The isolates originating from cultivars without the *Rlm7* resistance gene were mainly identified as *L. maculans*, whereas the isolates obtained from cultivars harbouring *Rlm7* resistance genes were scarce and belonged mostly to *L. biglobosa*.

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

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

Our work was focused on protein hydrolysates

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

**Acknowledgements** This research was supported by grant MŠMT COST LD14056.

## **Blackleg distribution, severity and chemical control**

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

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

pycnidial isolates (243) were obtained from leaf lesions and pathogen identification was done by morphology on PDA and will be confirmed by species-specific PCR. Changes in the frequencies of avirulent *AvrLm1*, *AvrLm4* and *AvrLm7* alleles in *L. maculans* populations at different sites in the UK are being investigated by inoculation of conidial suspensions on the cotyledons of a differential set of cultivars (Balesdent *et al.*, 2005). The molecular events leading to virulence against *R* genes that are currently used (*Rlm1*, *Rlm4* and *Rlm7*) will be analysed by exploiting the *L. maculans* genome sequence (Rouxel *et al.*, 2011) and *Brassica napus* genome sequence (Chalhoub *et al.*, 2014) data.

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

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

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

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

compare the expression of genes associated with the defence response.

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

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

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

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**Abstract:** In the Czech Republic, the increase in area sown with oilseed rape during the last two decades has been contributing to the increased appearance of a worldwide economically important disease of oilseed rape – phoma stem canker. Phoma stem canker is caused by two closely related fungal pathogens, *Leptosphaeria maculans* and *L. biglobosa*. The objective of this study was to assess the countrywide distribution of these two causal agents of the disease and their occurrence in oilseed rape tissues. In the growing seasons from 2007 to 2011, 1454 leaf samples with spots were visually identified based on symptoms and then analysed using species-specific PCR. Out of these, 39 and 15 % were detected as *L. maculans*- and *L. biglobosa*-infected, respectively, in case of single species-infected samples, while 26 % corresponded to the co-infection by both species. DNA of either one or both of *L. maculans* and *L. biglobosa* was not detected in 20 % of the leaf spot

samples. Furthermore, some isolates, that were collected from selected leaf spots and maintained in pure cultures, were identified based on pigment production during culturing on solid and in liquid media and PCR assay. In this case, the co-infection by *L. maculans* and *L. biglobosa* in a single leaf spot appeared as well. In years 2007–2012, 708 bases of oilseed rape plants divided into upper stem, lower stem, root collar and root parts were analysed using symptom identification and PCR. The proportion of plants in which *L. biglobosa* DNA was amplified was greater than that of plants with *L. maculans* DNA and 40 % of tested plants were found to be co-infected by both *L. maculans* and *L. biglobosa*. According to our results, it appears that *L. maculans* is the predominant species in autumn, while *L. biglobosa* is more successful species than *L. maculans* in colonization of oilseed rape tissues in later growth stages of a plant in conditions of the Czech Republic.

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

**Acknowledgements** This work was financially supported by the Ministry of Agriculture of the Czech Republic within the framework of The National Agency for Agricultural Research, projects No. QJ1310227 and QH81127.

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

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

There were differences in the effectiveness of the two fungicides in control of phoma leaf spotting between the cultivars. Prothioconazole reduced the severity of *L. maculans* phoma leaf spots on two cultivars and *L. biglobosa* phoma leaf spots on three cultivars. Penthiopyrad + picoxystrobin reduced the severity of both *L. maculans* and *L. biglobosa* phoma leaf spots on three cultivars.

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

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

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**Abstract:** The purpose of the current study was an estimation of the influence of application of plant growth regulators (PGR) and fungicides on growth of oilseed rape (OSR), blackleg disease development, plant wintering and plant yield. A multifactorial field experiment with 4 replications was designed in split plots in Ahlum, Lower Saxony, Germany from 2012/2013 to 2014/2015. The plots consisted of 4 OSR cultivars (Elektra, Vitara, PR 46W20 and Genie) and subplot treatments were 5 different PGRs (Ampera, Carax, Folicur, Tilmor and a combination of Imbrex/Folicur) which were applied twice; in autumn (BBCH 14–18) and in spring (BBCH 30–55). Observations such as growth parameters like plants/m<sup>2</sup> and plant height, yield parameters like number of pods/plant, number of seeds/plant, thousand grain weight, seed

yield and also evaluation of Phoma disease severity were taken using EPPO standard procedures. The data of the individual parameters were evaluated separately for each year of the field experiment. PGRs application effect on plant survival during winter was not observed for treatments. In contrast, the application of PGRs and fungicides had a substantial influence on the control of Phoma leaf symptoms in autumn. Hereby, Phoma leaf disease in autumn was not significantly correlated with OSR seed yield. In contrast, Phoma stem canker at growth stage 81–83 had a negative correlation with TGW and yield. The plant height at harvest stage varied significantly due to the different treatments. Among all tested yield factors, only number of plants/m<sup>2</sup> and pods/plant had a significant effect on OSR TGW and seed yield.

**Keywords:** *Brassica napus*, *Phoma lingam*, yield parameters, disease severity



## **Non-chemical control of diseases**

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

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

The genus *Trichoderma* proved great potential for biocontrol in many crops and the first attempts to control rapeseed diseases were also very promising.

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

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

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

The study assessed the influence of antagonistic fungi on the growth of *Sclerotinia sclerotiorum* isolates. Five cultures of antagonistic fungi were used: *Trichoderma* sp., *T. viride*, *T. harzianum*, *Ulocladium atrum*, *Coniothyrium minitans* and four *Sclerotinia sclerotiorum* isolates. The isolates were inoculated on a potato dextrose agar (PDA) in Petri plates (9 mm) by placing rings (5 mm) of the mycelia of the pathogen and antagonist at a distance of 6 cm from each other. Isolates of the pathogens and antagonists inoculated individually on a PDA were used as control samples. The experiment was conducted

at a temperature of 21 °C. During the following days of incubation the influence of the antagonists on the growth of *S. sclerotiorum* was assessed by measuring the radius of the fungal colonies and comparing it with the values observed in the control samples.

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

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

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**Abstract:** The agrotechnical method belongs to the methods used in the integrated crop protection of winter oilseed rape (OSR) against fungal diseases. Crop rotation remains one of its underestimated components, especially when it comes to keeping long enough intervals between growing crops of the same group. Some of the best forecrops for OSR are the different types of legumes. However, for the natural and economic reasons, OSR is usually grown after cereals. The higher the OSR share in crop rotation, the higher the risk of pests, including fungal diseases. During the growing seasons of 2012/2013 – 2014/2015, the experimental field at the Plant Protection Institute – National Research Institute, Sosnicowice Branch (south-western Poland) and the Field Experimental Station in Winna Góra (mid-western Poland) were the sites of controlled plot trials. Winter OSR Visby F1 was grown in three different crop rotation schemes: 3–4 years after OSR, after wheat and in monoculture. To protect the crop against *Sclerotinia sclerotiorum* and pod diseases, the researchers selected three fungicides, approved for use in Poland, based on active substances that belong to different chemical groups (prochloraz, azoxystrobin, dimoxystrobin+ boscalid) and applied them at three different timings, i.e.: at the beginning of flowering (BBCH 60–61), full flowering (BBCH 64–65) and end of flowering (BBCH 69–70). The trials were designed to assess the ef-

fect of crop rotation and fungicidal treatments used at the flowering stage on fungal diseases in OSR. At the stage of pod formation, researchers assessed the percentage of plants infected with *S. sclerotiorum*. At the same time, they analyzed the pod infection and the findings were presented as % of infected pods. The trial also assessed the seed yield and TGW. The findings underwent statistical analysis. Statistical calculations were performed using the Statistica 8.0 software and ANOVA analysis of variance. The results were compared using Duncan's test with significance level of  $P < 0.05$ . The results indicate that the trial location, crop rotation and treatment timing had an effect on occurrence of fungal diseases. OSR infection was highest in monoculture and second highest in crop rotation when OSR followed wheat. Infections increased with the increasing share of OSR. In crop rotations with higher OSR share, treatments applied at the stage of full bloom worked best against the different diseases, especially *S. sclerotiorum*. Seed yield was tied to the crop rotation scheme and the timing of fungicidal treatment. Due to the different growth rate of fungal diseases, the most effective timing for treatment applied at the flowering stage in mid-western Poland was the beginning to full blooming of OSR (BBCH 61–65), whereas for the south-western Poland, it was the from the full bloom until the end of blooming (BBCH 64–70).

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

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

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

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

In both tillage systems the same protection measures were used against pests (TFI value). As far as the second factor is concerned, the level of protection varied and the total TFI value (the sum of herbicide and fungicide) was as follows:  $b_1 = 1.97$  and  $b_2 = 1.0$ . The following broad-leaved weed species were predominant: *Centaurea cya-*

*nus*, *Geranium pusillum*, *Viola arvensis*, *Matricaria inodora*, *Anchusa arvensis* and grass weed species: *Elymus repens* and *spica-venti Apera*. In the simplified tillage system weed infestation was significantly higher than in the ploughing system.

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

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

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

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**Abstract:** Reports of light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, in commercial winter oilseed rape crops in Great Britain have been increasing in Scotland, England and Wales. In Scotland, 62% of crops were reported to have light leaf spot symptoms in spring 2015, and in England and Wales 85% of crops were affected. Yield losses caused by light leaf spot in England and Wales were estimated to be in the region of £140 million (£178 million) despite the majority of oilseed rape crops receiving at least 3 fungicide applications in a single season. Control of light leaf spot is predominately through the application of two spray fungicide programmes, with the first usually in November followed by another fungicide application pre-/at stem extension onwards (usually February/March) and using varieties with disease resistance. Growers in the UK can select varieties based on a range of

characteristics described in the Recommended Lists® for cereals and oilseeds which are published annually by the Agricultural and Horticultural Development Board (AHDB). Independent information on efficacy and appropriate fungicide doses for disease control including light leaf spot for a range of fungicide modes of action are also available from the AHDB, however, despite the integration of strategies to control crop diseases being encouraged, there is relatively little information available on the benefits such strategies can offer growers. This paper examines data derived from experiments using combinations of varietal resistance and fungicide application timing for light leaf spot control in 2015 from Scotland and England. The benefits of using integrated control strategies for control of light leaf spot, as well as the potential impact for control of other diseases, will be discussed.

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

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**Abstract:** *Sclerotinia* stem rot is responsible for the most severe attacks on oilseed rape in France, and therefore, is controlled by 1-2 sprays of fungicides. The use of SDHIs (succinate dehydrogenase inhibitors), mostly represented by boscalid, led to the selection of low to high resistant strains, as measured *in vitro*. This resistance evolved and spread in French

populations of *Sclerotinia sclerotiorum*. It was systematically correlated in our sampling with one of the seven changes detected in the subunits B, C or D of the target enzyme of SDHIs using molecular tools. Although reduced field performance is infrequent, recommendations for growers are provided to deploy appropriate resistance management strategies.

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

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

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**Abstract:** Pasmo is a major disease affecting linseed. Infected linseed stubble were observed during the autumn, showing not only the presence of pycnidia of *Septoria linicola* but also more

pseudothecia, indicating the existence of the teleomorph *Mycosphaerella linicola* in France, potentially responsible for primary contamination of winter linseed.

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

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

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**Abstract:** Oilseed rape is the most widely cultivated crop species in the *Brassicaceae* family today and has now become the third leading source of vegetable oil and oil meal in the world. The acreage of cultivated oilseed rape plants is increasing yearly. In Poland, oilseed rape is the most important crop grown for industrial purposes, and there is potential for increasing the cultivation area of this crop. Nowadays, to obtain the highest possible yield, it is important to ensure growth conditions close to optimal. In the field, plants are often exposed to several stressful conditions therefore using growth stimulation programs and optimal nutrition to improve plant status has become quite common practice. The aim of this work was to study the effect of different nutrition and growth stimulation programs on fluorescence of chlorophyll a and gas exchange efficiency in leaves of oilseed rape. Oilseed rape cv Saveo F1 (Syngenta Seeds) plants were cultivated in the 2015/2016 growing season at Ksiaz Wielkopolski in Poland. Biostimulant Dynamic Cresco 0.8 l/ha was applied as a single foliar spray. Two different 'optimal' nutrition programmes were also tested

(Opti-1 and Opti-2). The following parameters of intensity of transpiration were measured using an infra-red gas analyser method (Photosynthesis System Licor 6400X): intensity of photosynthesis (Pn), stomatal conductance (gs), intercellular CO<sub>2</sub> concentration (Ci), transpiration rate (Tr), water use efficiencies (Pn/Tr), RuBP carboxylation limitation (Pn/Ci) and relative chlorophyll content (Chl). Chlorophyll a fluorescence was recorded with Handy Pea (HansaTech Inc.). The efficiency of excitation energy capture by open PS II reaction centers (Fv/Fm) and the quantum yield of electron transport at photosystem II were determined.

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



## Clubroot of oilseed rape

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

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**Abstract:** Clubroot disease, caused by *Plasmodiophora brassicae* (Wor.), has been spreading on winter oilseed rape (*Brassica napus* L.) in the Czech Republic over the past five years. Research on *P. brassicae* in the Czech Republic is therefore important for the development of effective strategies to manage clubroot under Czech environmental conditions. Clubroot infestation and spread were monitored over five years and a map of infestation were created. Experiments with clubroot resistant cultivars of winter oilseed rape were carried out in the field and greenhouse. In the greenhouse, six clubroot resistant cultivars were grown in infested soil collected from various fields in the Czech Republic, and assessed for disease severity. The

soil samples were also tested for the presence and amount of *P. brassicae* inoculum by conventional and quantitative PCR analysis. In the field experiment, seven clubroot resistant cultivars were grown and disease development was monitored monthly. Yields were measured at the end of the cropping season. Finally, a set of 17 *P. brassicae* field isolates from across the Czech Republic were assessed for pathotype designation on the differential hosts of Williams, Somé *et al.*, and the European Clubroot Differential set. Collectively, the information obtained on the effectiveness of host resistance and pathogenic diversity of *P. brassicae* populations from the Czech Republic may help to more effectively manage clubroot in this country.

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

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

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

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

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

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

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**Abstract:** Clubroot, caused by *Plasmodiophora brassicae*, is a major disease of cruciferous crops that is widely dispersed through the world. The oilseed rape production area in Germany is roughly 1.5 million hectares per year and oilseed rape remains an important crop in the rotation. Nowadays, clubroot disease is an increasing problem not only to oilseed rape but also to all Brassica species. The detection of nearly 50 new *P. brassicae*-infested fields during 2012–2015 across several federal states in Germany suggests that clubroot disease maybe more widespread in oilseed rape fields than previously thought. To date, growing resistant cultivars is the most effective and environmentally safe strategy for controlling clubroot (Hirai 2006; Diederichsen *et al.* 2009), but sometimes this resistance can be overcome as new pathotypes of the pathogen emerge. In the present study, field trials with natural infection on three different locations in Germany were conducted in 2014 and 2015 to investigate control strategies for improving resistance in susceptible and resistant cultivars by evaluating the effect of different soil amendments at different times during

the growing season. Calcium cyanamide (300kg/ha; 50% calcium oxide) and burnt lime (150kg/ha) were applied to the soil surface one day prior to the sowing or when the oilseed rape plants had reached the growth stage (BBCH) 11–12. Soil moisture, soil temperature and soil pH at two different depths (15 and 30 cm) were measured at regular intervals over the growing season. Clubroot disease incidence and severity were assessed visually for the development of root galls. Field results in 2014 showed clear differences between the treatments. Changing the time of application had a significant effect ( $P \leq 0.05$ ) on the final severity of the disease. Relative to untreated controls, clubroot incidence and severity were decreased by application of fertilizer at later growth stages. In comparison with calcium cyanamide, burnt lime application has a smaller effect. In 2015, it was decided to increase the amount of burnt lime application to reach the standard field recommended amount. However, no infection or only low infections of *P. brassicae* were observed across field trials and there were no significant effects of any treatment on the incidence of disease.

**Keywords:** *Brassica napus*, clubroot, calcium cyanamide, lime, disease severity

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

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**Abstract:** Plants infected by *Plasmodiophora brassicae* are subjected to extensive change in developmental programs. During disease progression the pathogen needs to build an efficient feeding site, securing in this way successful production of resting spores. This developmental reprogramming is an outcome of interaction between the pathogen and infected host. During the disease progression we can observe change in growth regulator dynamics, altered patterns of cell proliferation and differentiation, increased cell expansion or eventual cell wall degradation as well as the redirection of nutrients towards the pathogen. Here we would like

to present recent progress in molecular and anatomical studies describing developmental changes occurring during the infection. With the help of an integrative biology approach we were able to describe mechanisms leading to change in cell fate determination triggered by *P. brassicae* in the infected host plant. We also found the link between developmental reprogramming and the usurpation of the host nutrition distribution system by *P. brassicae*. This work summarises the current state of knowledge on *P. brassicae* driven developmental reprogramming of plants and suggests ways to study exact role of observed changes in the host.

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

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**Abstract:** Blackleg is a disease of world-wide importance on oilseed rape (*Brassica napus*), which can cause serious losses in different countries. The disease is caused by closely related pathogens *Leptosphaeria maculans*, causing stem-base canker and *L. biglobosa*, causing upper stem lesions late in the growing season. In 2015, in some regions of Germany relatively late disease symptoms were observed on the upper part of stems, causing the crown canker which was responsible for lodging of the plants and yield losses. The results of morphology and species-specific PCR assays revealed that *L. biglobosa* constituted 58% of all isolates obtained from the infected stems. In general, besides growing resistant oilseed rape cultivars, fungicide application showed a significant reduction in the percentage of blackleg disease incidence and severity but little is known about the differences of sensitivity of *Leptosphaeria* spp. isolates to different fungicides. In the present study, the effects of the most important groups of fungicides (QoI, SDHI, DMI and MBC) were

examined on the germination of pycnidia and the inhibition of mycelial growth of 10 reference strains of *L. maculans* and *L. biglobosa*. Fungicide sensitivity tests *in vitro* were conducted using fungicide amended agar plates at 0.0, 0.001, 0.01, 0.1, 1.0, 10.0 and 100.0  $\mu\text{g a.s. mL}^{-1}$  concentrations. The results show that the two pathogens did not differ significantly in their growth rates under *in vitro* conditions. Lower concentrations of all fungicides (0.001, 0.01, 0.1 and 1.0  $\mu\text{g a.s. mL}^{-1}$ ) have no or a low effect on conidial germination and mycelial growth inhibition in either species. In contrast, at higher concentrations significant differences in growth inhibition were observed between *L. maculans* and *L. biglobosa* isolates when treated with fungicides. *Leptosphaeria maculans* isolates were significantly more susceptible to all fungicide groups than *L. biglobosa*. Even at the highest concentrations (100  $\mu\text{g a.s. mL}^{-1}$ ) none of the fungicides could achieve 50 % control of *L. biglobosa* in either the conidial germination test or the mycelial growth inhibition assay.

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

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

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**Abstract:** *Plasmodiophora brassicae* is the obligatory pathogenic protist causing clubroot disease in agriculturally important Brassica species. The disease has a detrimental effect on plants and leads to severe yield losses. The characteristic symptom of clubroot is the development of galls on the underground parts of the infected plants (Ludwig-Müller & Schuller, 2008). These structures resulting from hypertrophy and subsequent hyperplasy of cells (Malinowski *et al.*, 2012) are responsible for plant wilting and eventual plant death. The expansive gall growth is caused by the formation of giant cells which constitute final reservoirs of resting spores of the pathogen (Mithen & Magrath, 1992; Schuller *et al.*, 2014). Clearly, these changes observed during disease progression must be accompanied by massive cell wall modifications, which are typically regulated by cell wall remodelling enzymes e.g. cellulases, endotransglycosylases of xyloglucan and expansins. We believe that detailed characterisation of the cell wall remodelling process during *P. brassicae* infection will help to understand the mechanism used by the pathogen for invading plant cells and securing resting spore formation. Therefore, our research aims to identify the specific factors involved in the cell wall turnover process accom-

panying characteristic cellular responses observed during clubroot infection. In order to accomplish this task we will perform comparative proteomic studies by monitoring changes in cell wall protein profiles in galls (including giant cells) against appropriate non-infected controls. The experiments are based on the model plant *Arabidopsis thaliana* and involve the sequential cell wall protein isolation method (Feiz *et al.*, 2006) combined with 1-D and 2-D protein electrophoresis, followed by the mass spectrometry-based identification of proteins with differential abundance between treatments. It is highly likely that not only are structural features of cell wallchanging, but also some cell wall proteins that may act as signalling molecules during this process. These may include arabinogalactan proteins (AGPs) – a heterogenous group of proteins composed of polypeptide, glycan and lipid parts (Seifert & Roberts, 2007). In order to characterise their involvement, we are planning to use a series of commercially available antibodies for *in situ* detection within developing giant cells. Ultimately, we plan to generate transgenic lines with modified activities of the identified and selected cell wall proteins and test the potential impact of such modifications on *P. brassicae* pathogenesis.

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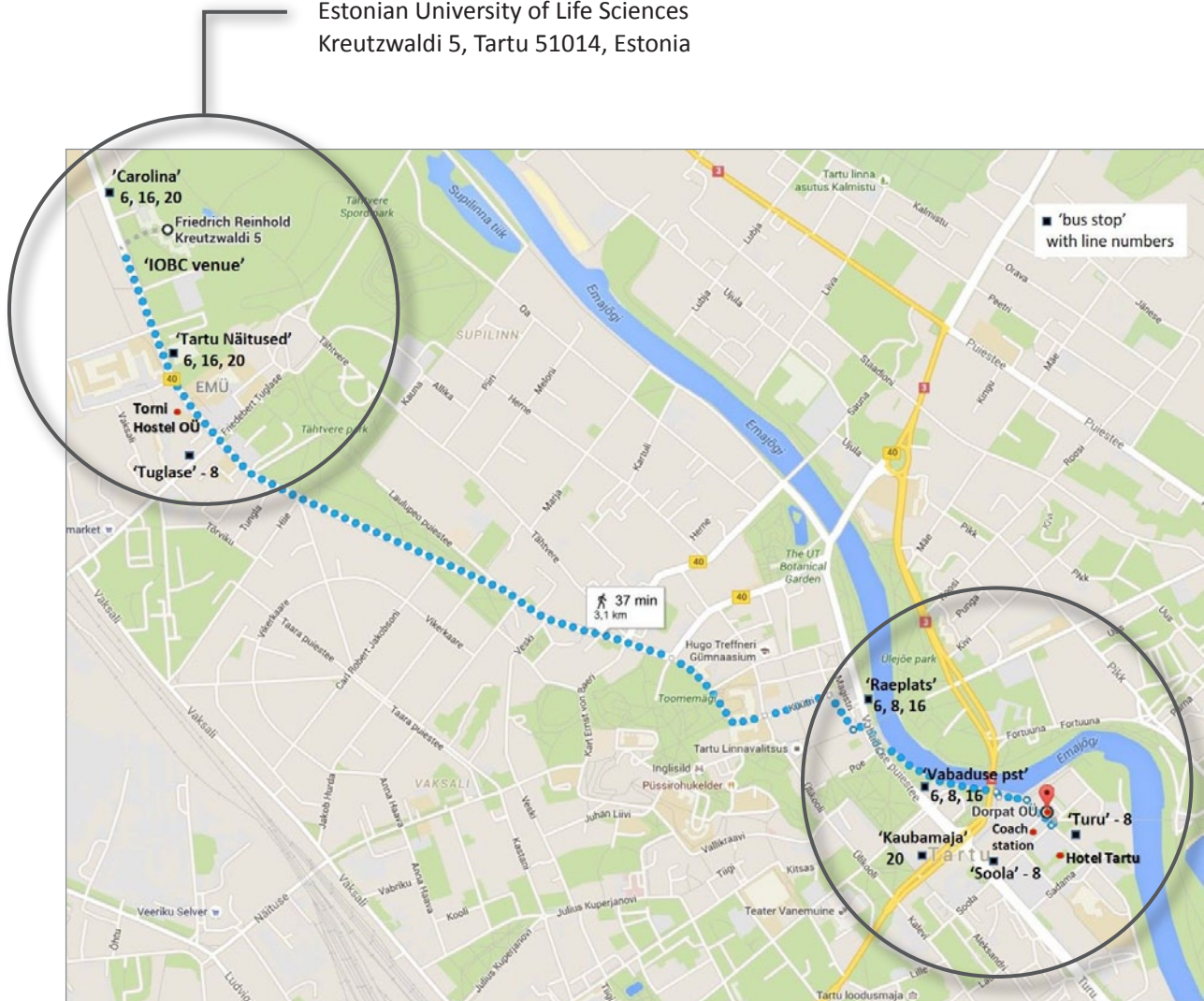


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