

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

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

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**Samantha M. Cook, Malgorzata Jedryczka, Ivan Juran  
and William Truman**

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## **Welcome at the Faculty of Agriculture of the University of Zagreb!**

The Faculty of Agriculture at the University of Zagreb has been dedicated, for almost 100 years, in the education of agriculture and its related sciences; to producing highly qualified experts and developing and broadening professional knowledge in this area. In addition to teaching, scientific research is a fundamental activity undertaken in pursuit of our mission – the improvement of the Croatian agricultural sector. Our faculty holds a leading position in Croatian agricultural science due to success of the large number of scientists, scientific projects and publications.

Plant protection and ‘phytomedicine’ takes a very important place in the University studies at the Faculty of Agriculture at all three educational stages: at Undergraduate level, and via Graduate and Postgraduate studies; but also in our other activities connected to life-long learning, and services which support the private sector of Croatian agriculture.

In our work we strongly support all activities of our scientists, teachers and students in international cooperation. On behalf of this, I am pleased to thank you for your participation in this meeting.

I am delighted to welcome all 60 participants of this meeting who come from 11 different European countries to the 17<sup>th</sup> IOBC Working Group Meeting on Integrated Control in Oilseeds (ICOC) which we are proud to host in our Faculty. I hope you are going to have successful discussions on your topics regarding the current problems encountered in the production, entomology and plant pathology of oilseed crops in Europe.

I believe that this meeting will be a stimulus for all of us to establish further cooperation on improving agricultural production with greater efficiency of plant protection.

Dean of Faculty  
Prof. Zoran Grgić, PhD



## Preface

The IOBC Working Group on Integrated Control in Oilseeds (ICOC) will meet for the 17<sup>th</sup> time in Zagreb, Croatia, September 18-20, 2018, to discuss the current problems encountered in the production of oilseed crops in Europe. As we have already noticed in previous years, the problems observed by us in Europe are very similar to those reported in the other parts of the world: problems with pests and diseases, resulting in crop damage and yield loss.

The meeting will comprise both joint and separate sessions devoted to entomology and plant pathology of oilseed crops, with the final get-together summarizing our respective discussions. In such a way, we can discuss the technical and experimental details of our work in subgroups of specialists, but also share the most current knowledge on problems in oilseed production with the specialists of the other sub-group. The result – we are double winners, as we can dwell on very detailed aspects of studies that interest us directly, without losing much of the general picture.

As usual, the main crop to be discussed is oilseed rape (*Brassica napus*) – an amazingly versatile crop grown on all continents, from Europe through Asia to North America and Australia. Europe is still the world's leader with 21.7 million tons harvested in 2015 representing nearly 70% of global production (EuroStat). However, the leading position may be lost, if we do not manage to counteract the yield losses due to increasing pest damages.

This year entomologists will dwell on the increasing pressure on insecticidal active ingredients; on one hand the continued development of pest resistance to available compounds and on the other, revocation of the neonicotinoid seed treatments by the EU. Entomologists like to joke that pathologists don't need to worry about clubroot if farmers live in a hotspot area for cabbage stem flea beetle, as these pests can completely devastate the crop before proper establishment. But it is no joke that many farmers (particularly in UK) are giving up growing oilseed rape due to these problems. We must work together to develop sustainable, alternative control strategies for insect pests of oilseeds that minimize insecticide use and reduce the risk of resistance.

Current problems discussed by plant pathologists primarily concern clubroot of oilseed rape caused by the microorganism classified as belonging to the Kingdom of Rhizaria. Ever heard of this Kingdom? If not, please read in this volume of the IOBC Bulletin about the havoc it can cause. Problems with the stem canker of oilseed rape have partially been overcome by the introduction of the *Rlm* resistance genes and the current step is to combine them with quantitative resistance to biotic and abiotic stress. Also, new molecular techniques will be presented to enable quantification of the pathogens in plant tissues as well as in the soil. But the pathogens 'never sleep'; new races, pathotypes, lineages as well as resistances to current chemistry and resistance genes are regularly found which pose new threats to oilseed rape production.

Against this background, both sub-groups discuss methods for ecology-based IPM strategies that can be realized and that are currently viewed as a need, not just an option!

This year we will also meet the newcomer to our group; hemp. Plant breeders obtained dioecious and monoecious hems, free from hallucinogenic compounds, but producing big quantities of seeds with interesting oil composition. As usual, however, these oilseeds also have many enemies, among them insects, fungi, bacteria and viruses. And they are loved by birds!

Similar to our previous meetings, this meeting will include nearly 60 participants from 11 countries, including Austria, Croatia, Czech Republic, Denmark, Estonia, France, Germany, Poland, Sweden, Switzerland and the United Kingdom. So many people and countries, but we mostly know each other well. It is not an exaggeration to say we are one big family. As usual in any family, one can expect hot discussions and many *different* views on the same topic... but this is why we meet! Surely, it will not be boring!

Gosia Jedryczka – Working Group Convenor,  
Sam Cook – WG sub-convenor,  
Ivan Juran – the Local Organizer,  
Giselher Grabenweger – the Liaison Officer of the IOBC-WPRS



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# **Opening plenary lectures**



## **An introduction to IOBC and ICOC**

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The International Organisation for Biological Control (IOBC) promotes the research and development of environmentally safe and effective methods of pest and disease control. Focus is on biological control and its implementation within integrated pest management (IPM). IOBC was established in 1955 as an organisation of institutional members as well as individual scientists working in all disciplines relating to biocontrol. Since then, it has become a well-known, non-profit organization, providing independent and professional advice on biological control and IPM to farmers and advisory services as well as to policy makers and governments.

One of IOBC's missions is to promote international cooperation in research and development and to facilitate the transfer of scientific knowledge into agricultural practice. This requires a regionalized organisation and close collaboration of all stakeholders. In order to achieve this goal, IOBC is organized in six regional sections, each of them running an array of specific working groups.

IOBC-WPRS (West Palearctic Regional Section) currently comprises 20 working groups (WGs) focussing on specific crops (e. g. citrus, olives, viticulture, fruit crops, oilseed crops, vegetables), pest organisms (e. g. mites, plant pathogens), and methods (e. g. plant resistance breeding, application of pheromones and semio-chemicals, landscape management). WGs usually consist of about 30 to more than 100 members, including scientists, students, and representatives of governmental institutions, advisory services and the biocontrol business. Meetings take place every second or third year to help exchange recent scientific findings, draw attention to newly emerging plant protection issues, or share experience from laboratory and field experiments. Lively discussions and excellent networking opportunities contribute significantly to the popularity of IOBC-WPRS WG meetings.

The IOBC-WPRS WG "Integrated Control of Oilseed Crops" (ICOC) has been active since 1982. It was founded by a group of oilseed rape pathologists and entomologists, and after some years expanded its activities to other oilseed crops including sunflower, linseed and false flax. The WG has become an international forum for the development of sustainable low-input oilseed crop production systems, with the aim of fostering biological and integrated control in oilseed crops, to minimize pesticides and fossil fuel use, and to maximize the exploitation of genetic resources.

The conference in Croatia's capital Zagreb is the 17<sup>th</sup> meeting this relatively small but very active WG. In a world of growing concern about the risks of chemical pesticides, and at the same time growing interest in alternative control strategies, it is the right time for ICOC to develop collaborative research with pathologists and entomologists working together to push integrated control in oilseed crops another step forward!



## **Rapeseed production in Croatia and future potential for area and yield increase**

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### **Production analysis**

After sunflower, rapeseed is the second most produced oil plant in Europe today. The EU member states produce 21.2 million tons of rapeseed (2012-2016 average), which is 30.3 percent of the total global production (FAOSTAT, 2018). According to the Croatian Bureau of Statistics, total rapeseed production in Croatia varied from 19,996 to 112,990 tons over the last 15 years, showing a mild uptrend. In the same period, rapeseed area grown varied between 8.413 to 36.778 ha, and seed yields ranged from 1.84 t/ha (2003) to 3.10 t/ha (2014 and 2016). This result is not satisfactory, since in agriculturally developed countries (e. g. Germany) average seed yields are over 3.8 t/ha and grown on much larger areas.

It is undisputable that areas under oil crops should be enlarged in Croatia because domestic production of oil crops (sunflower, soybeans, rapeseed) cannot satisfy the demand of our vegetable oil factories for this raw material. Areas under rapeseed should be increased especially in northwestern Croatia where ecological conditions for its production are most favourable (Pospišil *et al.*, 2011). Areas under rapeseed can be further increased by re-cultivating deserted and uncultivated areas. Total rapeseed production can also be increased by improving average yields to 3.5-4.0 t/ha; both ecological and technological conditions exist to make this possible. Technological problems primarily include: precise and efficient fertilization, optimal sowing time for each hybrid, optimal plant density and crop protection.

### **Critical review of production technology**

Although average yields of rapeseed seed have been increased in recent years, they are still variable despite relatively favourable agroecological conditions, which points to certain shortcomings of the production technology.

To achieve high yields of rapeseed, it is necessary to observe the basic agricultural management rules such as crop rotation and optimal time of applying cropping practices. Due to the hazards of excessive accumulation of pests and diseases, rapeseed should be grown in an 'adequate' crop rotation, returning to the same area only after 4-5 years (Pospišil, 2013).

There are some producers that inadequately prepare the soil for rapeseed, that is, apply only disking with heavy disk harrows or ploughing just before sowing. Several dry periods before and during sowing in recent years clearly demonstrated the adverse effects of such soil preparation. Sowing into freshly tilled soil results in un-uniform sowing depth and uneven emergence, unequal plant density and initial growth, are ultimately leading to a considerable decrease in plant density, and thereby also yield. Soil preparation for rapeseed should start

immediately after the harvest of preceding crop (mainly stubble cereals) by stubble cleaning, as well as early enough ploughing (at least three weeks prior to sowing) to a depth of 25-30 cm for the necessary settling of soil. The surface soil layer should be immediately crumbled and leveled after ploughing. Different ways of conservation tillage are currently used for rapeseed, such as soil crumbling without overturning, often combined with sowing in one or more runs. There are several types of machines for conservation tillage and/or simultaneous sowing of rapeseed. Use of rotary tillers is recommended because they intensify soil pulverization, thereby improving the soil water-air relationship, increasing water permeability and accumulation of precipitation water in deeper layers, and also because of appreciable energy saving per unit area. Such tillage systems for rapeseed are especially suitable in regions and years with arid conditions at sowing. However, without risking yield decrease, such systems are applicable only on fertile soils and with intensive use of mineral fertilizers and herbicides.

Proper supply of all nutrients, and particularly nitrogen, is a key prerequisite for increasing rapeseed yield. This is confirmed by researches on rapeseed that have recently been conducted in Croatia (Brčić, 2017; Spitek & Pospišil, 2017). Quantities and proportions of both main and micro nutrients as well as the time and method of their application should be determined according to soil analysis and the dynamics of uptake, namely, plant nutrient requirements during the growing season. Only in this way it is possible to rationally apply expensive fertilizers, since each pattern can lead to either a wasteful technology or particular nutrient starvation of plants, with consequent yield decrease and unstable production. Due to excessive soil moisture at the end of winter, rapeseed fertilization is often applied too late in some parts of the country with the consequence that the full effect of fertilizers is not realized. First fertilizer application should be done at the very beginning of spring growth, usually in the latter third of February, and the second application just before the intensive growth period, usually two weeks after the first application. Rapeseed nitrogen requirements are the highest in this short period, amounting to over 120 kg N/ha (for yields over 3.5 t/ha). Further possibilities of increasing rapeseed yield involve the control and application of secondary nutrients and micronutrients, notably sulphur and boron.

Good and timely sowing are among the key prerequisites for efficient and stable rapeseed production. The beginning of September (1-10 Sept.) is the optimal sowing time for winter rapeseed in this production region. Delay leads to yield decrease. Maximum yielding potential of modern rapeseed cultivars can be achieved only in the growing area of ideal size and shape for each cultivar. Plant density of 35-45 plants/m<sup>2</sup> at harvest is optimal for currently most produced hybrids and that of 50-70 plants/m<sup>2</sup> for line varieties. Due to the specificity of currently prevalent cultivars in Croatia, the sowing technology has to be harmonized with the particular characteristics of each cultivar.

Climate change may require further adjustment of rapeseed production technology by altering the need for cultivars, sowing/harvesting time, tilling methods, fertilizer application, pesticides, etc. to match the changing conditions

**Key words:** rapeseed, production technology, yield

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## **Resistance monitoring of the most important economically harmful organisms in Croatia – national programme**

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### **Introduction**

There are permanent needs to increase agricultural production worldwide to feed the world's increasing population. Today's agricultural production largely relies on the use of synthetic inputs, although partly due to public pressure more attention is being paid to various forms of sustainable agricultural production (Lélé, 1991). Consequently, despite the great advantages of using synthetic plant protection products in agricultural production, there is a need for the development of new technologies that could partially replace them and reduce their adverse environmental impacts. The most common causes of resistance are: frequent use of a plant protection product and/or use of products containing active ingredients with the same or similar mode of action, utilization of reduced dose in relation to the recommended rate, absence of non-chemical protection measures etc.

### **National Programme**

This Programme is based on the National Plant Health Act, the Act of Sustainable Use of Pesticides, and Guidelines on the establishment of an action plan for the sustainable use of pesticides and Directive 2009/128/EC of the European Parliament. The Croatian Ministry of Agriculture issued a strategic document: the National Action Plan for Pesticide Sustainable Use (NAP) for the period 2013-2023 in accordance with the requirements of Directive 2009/128/EC (EC, 2009). The NAP provides for the implementation of the Guidelines on the establishment of a framework for the sustainable use of pesticides with a view to reducing the risks to humans and other animals associated with the use of pesticides and to promoting integrated and alternative measures to control harmful organisms in agriculture.

#### ***Aims and structure of the Programme***

The main objective of the Program is to establish a systematic monitoring of the emergence of pesticides resistant organisms to plant protection products at national level for the period 2017-2020.

Specific objectives are to:

- review the recent research on the occurrence of harmful organisms that have become resistant to plant protection products in the Republic of Croatia,
- sample and test the sensitivity of the most important economic insect pests (*Tribolium castaneum*, *Rhizopertha dominica*, *Panonychus ulmi*, *Aphis fabae*, *Tuta absoluta*, *Bothynoderes punctiventris*, *Chaetocnema tibialis*, *Frankliniella occidentalis*,

*Trialeurodes vaporariorum*, *Bemisia tabaci*, *Leptinotarsa decemlineata*, *Oulema* spp., *Brasicogethes aeneus*) to different groups of insecticides, where there is a high risk of occurrence of resistance (i. e. are regularly controlled by conventional insecticides in conventional and integrated agricultural production),

- Sample and susceptibility-testing of the most important pathogens; the casual agents of plant diseases (*Botrytis cinerea*, *Uncinula necator*, *Monilinia fructicola*, *Cercospora beticola*, *Phytophthora infestans*) to different fungicidal groups where there is a high risk of resistance (i. e. are regularly controlled by synthetic fungicidal application)
- Sample and test the susceptibility of the most important weeds (*Amaranthus retroflexus*, *Sorghum halepense*, *Erigeron canadensis*, *Papaver rhoeas*, *Chenopodium album*, *Ambrosia artemisiifolia*, *Echinochloa crusgalli*) to different herbicide groups where there is a high risk of occurrence of resistance (i. e. are regularly controlled by synthetic herbicides in conventional and integrated agricultural production,
- Createa database on the appearance of resistance and degree of resistance of pests to plant protection products,
- Create anti-resistance strategies to prevent or limit the development of pesticide resistance for the most endangered crops in Croatia,
- Education of producers e. g. via production of popular leaflets and brochures with research results.

**Key words:** resistance monitoring, national programme; Croatia

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## **Joint Session – General papers**



## **An overview of pathogen and insect threats to fibre and oilseed hemp (*Cannabis sativa* L.) and methods for their biocontrol**

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**Abstract:** Hemp (*Cannabis sativa* L.) is a treasure trove plant for both sustainable agriculture and industrial usage. It has multi-application properties due to the production of fibre and high quality oil, biomass as a safe source of energy, and numerous compounds for the pharmaceutical sectors, including mainly cannabinoids (THC and CBD) which have a wide range of psychotropic activities. Hemp, like other plants, suffers from a wide range of pests and pathogens. They cause plant damage, huge annual loss of biomass and seed yield as well as the reduction of the quality of the products. With increased demand for hemp products, its production area is anticipated to expand greatly; previously developed tolerance of environmental pressures or defense mechanisms against biotic threats may not meet the demands of new environments and the additional pathogens that will be encountered. In this review we focus on the most common fungal, oomycetes, viral and pest diseases attacking hemp both worldwide and in Poland. We also highlight the methods of biological control that make possible the maintenance healthy plants as well as the high quality of hemp products.

**Key words** *Cannabis sativa*, oilseed hemp, pathogenic fungi, pests, integrated control

### **Introduction**

*Cannabis sativa* L. is one of the earliest domesticated plant species (Schultes *et al.*, 1974) originating from central Asia (McPartland, 2000). Cannabis has been used for millennia as a source of fibre, oil and as a folk medicine. This valuable crop was introduced to Poland in 1946, where the breeding of hemp was mainly performed at the Institute of Natural Fibres in Poznan. The first dioecious varieties included: LKCS D, Szelejewskie, Pustkowskie, Sieleckie Południowe and Sieleckie Nowe (Spychalski, 2014). The maturation of male and female plants was shifted in time; so there was a demand for monoecious varieties that could provide plants of simultaneous maturity. These new monoecious varieties supplied the farmers with homogeneous fibre and they were also more suitable for mechanical harvesting. In Poland, the interest in growing hemp cultivars has increased from 100 ha in 2003 to more than 1,600 ha in 2008 (Grabowska *et al.*, 2009).

Cannabis is an attractive plant for both agriculture and industrial properties due to its multiple applications (Andre *et al.*, 2016). It is used in textile and paper industry, in creation of construction materials, as well as the automotive, energy, cosmetics, pharmaceutical and chemical industries (Grabowska *et al.*, 2009). Due to the great diversity of chemical

compounds found in hemp, it was dubbed “The Plant of the Thousand and One Molecules” (Andre *et al.*, 2016). The unique pharmacological properties of cannabis stem from the cannabinoids, a group of chemical compounds which mainly accumulate in female inflorescences with a wide range of psychotropic activities (Taura *et al.*, 2007a; Zirpel *et al.*, 2015).

Introducing any crop to a new environment is inevitably connected with the introduction of its enemies, including pests and pathogens. The Polish climate poses several challenges for agriculture and crop management: autumn rainfalls, creating high aerial humidity affect the pre and post-harvesting plant conditions and lead to several fungal diseases and the propagation of numerous pests. In order to maintain the vigor of the plant and the product quality, identifying and characterizing the diseases in each environment can assist in the optimization of crop protection strategies. In this review we focus on the most common diseases affecting hemp growth in Poland and define the best biological control which could be deployed to minimize the use of pesticides.

## **Morphology and taxonomy of hemp**

*Cannabis sativa* L. belongs to *Rosales* order, *Cannabinaceae* family with three species *C. sativa*, *C. indica* and *C. ruderalis* (Small & Cronquist, 1976). It has a diploid genome ( $2n = 20$ ) with a karyotype composed of nine autosomes and a pair of sex chromosomes (X and Y) (Schultes *et al.*, 1974). Mainly, hemp plants are dioecious but some monoecious cultivars have been obtained by plant breeders. In Poland, five varieties of monoecious fibre hemp have been cultivated: Bialobrzeskie, Beniko, Silesia, Tygra and Wielkopolskie. These varieties are adapted to the Polish climate and soil conditions (Grabowska *et al.*, 2009). Variety Henola is the first oilseed hemp variety and it is also unique due to its monoeciousness.

The morphology of the plant depends on the variety. The height of the traditional hemp plant ranges between 1.2 to 5 m with 4-20 mm stem diameter. The plants of cv. Henola are short, usually they range from 1.2 to 2.5 m, depending on the type of soil and season. Distinguishing the female and male plant in dioecious varieties is quite difficult before the flowering stage. Typically, the female plants are shorter with more branches compared to male plants (Clarke, 1999). The difference in the growth rates between males and females leads to uneven maturity at harvest because male plants mature more quickly than females (Berger, 1969). This problem has been overcome in monoecious varieties, including var. Henola, which produce very uniform plants.

## **Growth requirements of hemp**

The availability of nutrients, light and water are the major factors affecting growth of hemp. Traditional hemp cultivars can grow to a height of 5 m within 4-6 months (Clarke & Merlin, 2016). Vegetative growth is mainly during long-days whereas flowering and maturation occur under short-days. The critical day length for flowering is 12-14 hours depending on the variety (Sankari & Mela, 1998). Supplying fertilizers like nitrogen rapidly increases the growth of hemp, however too big a supply of nitrogen in some hemp crops can lead to leafy and succulent growth and increase the stem diameter above the optimum range (Ranalli, 1999).

## Common diseases of hemp

Despite the quote "hemp has no enemies" (Dewey, 1914) hemp, like other plants, encounters several types of fungal and pest diseases (McPartland, 1996). Different identification keys have been constructed for diagnostics of crop problems based on the prevalence of the diseases and symptoms. Some researchers use synoptic keys which rely on recognition patterns, while others prefer dichotomous keys with the structure of decision trees (McPartland, 2000). Table 1 shows the diseases occurring on hemp, caused by fungi and oomycetes. The list is based on the description of McPartland (2000) and Polish literature on this topic (Pietkiewicz, 1958; Czyzewska & Zarzycka, 1961; Zarzycka & Jaranowska, 1977) and our personal observations (unpublished). The list is made based on the pathogen names according to the taxonomy valid at the time of their detection. The survey contains only pathogens and does not describe ecto- and endo-mycorrhizal fungi.

**Fungal diseases on *Cannabis sativa* L.** include over 400 taxa of fungi (McPartland & Hughes, 1994). The diseases listed below are the most common in Poland.

***Botrytis cinerea* (grey mould)** has been reported as one of the most damaging fungi attacking both fibre and drug cultivars and causing grey mould, damping off and bud rot on hemp in Poland (Pietkiewicz, 1958). It affects the plants in both indoor and outdoor environments. Drug varieties are more susceptible to grey mould compared to fibre varieties (McPartland, 2000). Overwintering sclerotia raise spores in the following spring and the disease may be also seed-borne, which usually leads to the infection of seedlings. The inflorescence buds are more susceptible for fungal attack in drug cultivars, the usual symptom is a grey-white layer around the inflorescences (Scheifele, 1998). Stalk rot is more common in fibre cultivars; the fungus produces enzymes which can reduce the hardness of stems, subsequently they become soft and chlorotic (Patschk *et al.*, 1997). Biocontrol of grey mould has been achieved using *Trichoderma* species and *Gliocladium roseum*. Both genera can be applied by mixing with the soil. Additionally, bacterial species including *Pseudomonas syringae* and yeasts can be used as biocontrol for post-harvest control of the disease (McPartland, 2000).

***Fusarium*** is the name of the genus of filamentous fungi that parasitizes many crop and wild plants, it includes *Fusarium* wilts, blights, rots, and cankers (Ma *et al.*, 2013). The species *F. oxysporum* f. sp. *cannabis* and *F. oxysporum* f. sp. *vasinfectum* were reported as the causal agents of Fusarium wilt in Eastern and Central Europe, including Russia, the Czech Republic, Poland, and Romania since the middle of 20<sup>th</sup> century (Czyzewska & Zarzycka, 1961). The morphology of these two species is similar but their host range differs, as *F. oxysporum* f. sp. *cannabis* infects only hemp, whereas *F. oxysporum* f. sp. *vasinfectum* infects hemp, cotton, mung bean, pigeon pea, rubber trees, alfalfa, soybean, coffee, tobacco, and other plants. After overwintering of the chlamydospores in soil, hyphae start forming in the spring which invade, mainly, the xylem of host plants. These hyphae rapidly plug plant water-conducting tissues, causing wilt and damping off in the seedlings (Pietkiewicz, 1958). These symptoms appear as dark spots and chlorosis of lower leaves, followed by wilting and upward curling of leaf tips. Infected stems turn yellow and inside the xylem there is a reddish-brown discoloration. Symptoms caused by *Fusarium oxysporum* are similar to wilt caused by *Verticillium* wilt and Texas root rot, Southern blight and *Rhizoctonia*, sore shin (McPartland, 2000). It is very difficult to find effective fungicides to combat *Fusarium* species, reported biocontrol measures include the mixing of *Trichoderma lignorum* into soil (Czyzewska & Zarzycka,

1961). Commercial biocontrol treatments contain *Burkholderia cepacia* and *Streptomyces griseoviridis*.

**Leaf diseases** are mainly reported in fibre cultivars as well as in *C. ruderalis*, the species *Septoria cannabis* and *S. neocannabina* were reported as the casual agents of yellow leaf spot disease (Szembel, 1927). The overwintering form is pycnidia present on crop residues near the soil surface. Spores are released in June and the infection appears as small white-yellow lesions on the lower part of the leaf which dry out and fall apart leaving holes in leaves (Nykter, 2006). Other diseases causing serious damage to leaves, reported by McPartland (1995), brown leaf spot (*Phoma* and *Ascochyta* species) and pink rot (*Trichothecium roseum*). Additionally, brown blight caused by *Alternaria* spp. and *Stemphylium* species, anthracnose caused by *Colletotrichum* species and white leaf spot caused by *Phomopsis ganjae* are regarded as important causes of leaf damage (McPartland, 1983). Some fungal species can also infect stems, especially *Trichothecium roseum*, *Phoma*, *Stemphylium*, *Colletotrichum*, and *Phomopsis* species. No biocontrol against fungi causing leaf spots is currently available.

*Alternaria* is a genus of saprophytic, ascomycete fungi including nearly 300 species (Lawrence *et al.*, 2012). In Poland these fungi are known for causing leaf necrosis with dry spots on hemp leaves (Zarzycka & Jaranowska, 1977). The fungus remains dormant until spring, spores are produced abundantly when the temperature rises to 25-30 °C. Spores are dispersed by rain droplets and wind and they usually initiate disease symptoms on the lower parts of plants, where the canopy maintains higher humidity. Spores start germinating and penetrate the leaf epidermis within 12 hours of contact (McPartland, 2000). This fungus may also attack the stem of young seedlings creating grayish-brown spots and causing rot which kills the host plant. Fungal species belonging to the genus *Alternaria* causes 20% of total agricultural spoilage, in addition to causing allergic reactions for humans, associated with fever, asthma and skin problems (Lawrence *et al.*, 2013). Control against dark spot entails proper plant hygiene, good ventilation and avoidance of over-watering. Furthermore, natural fungicides, such those based on horsetail (*Equisetum arvense* L.) can be applied.

**Powdery mildew** is a common fungal disease infecting a wide range of crops. The species *Leveillula taurica* f. sp. *cannabis* and *Sphaerotheca macularis* were reported in Eastern Europe as a casual organism for powdery mildew in hemp (Jaczewski, 1927). It arises in temperate and subtropical regions infesting outdoor hemp (Transhel *et al.*, 1933). The early signs of infection appear on the upper leaf surfaces as dust as the fungi raises powdery mycelium, the leaves become chlorotic and turn to necrotic before dying. It is possible to detect the disease at earlier stages, when it appears as small white ‘cushions’ on leaves. For biocontrol, hyperparasitic fungi such as *Ampelomyces quisqualis*, a strain of *Verticillium lecanii* and the fungus *Sporothrix flocculosa* have been used in other crops to protect them from powdery mildew (Kendrick, 1985).



Table 1. Diseases of hemp caused by fungi and oomycetes worldwide and in Poland, listed in the scientific literature

Name of the diseases	Causal Organism
<b>Kingdom: Fungi</b>	
Grey Mould	<i>Botrytis cinerea</i> , <i>Botrytis infestans</i>
Yellow Leaf Spot	<i>Septoria cannabis</i> , <i>S. neocannabina</i>
<i>Rhizoctonia</i> Sore Shin & Root Rot	<i>Rhizoctonia solani</i> ( <i>Thanatephorus cucumeris</i> )
<i>Fusarium</i> Stem Canker	<i>Fusarium sulphureum</i> , <i>F. graminearum</i> , <i>F. lateritium</i> , <i>F. sambucinum</i> , <i>F. avenaceum</i> , <i>F. culmorum</i>
<i>Fusarium</i> Foot Rot & Root Rot	<i>Fusarium solani</i>
<i>Fusarium</i> Wilt	<i>Fusarium oxysporum</i> f. sp. <i>vasinfectum</i> , <i>F. oxysporum</i> f. sp. <i>cannabis</i>
Powdery Mildew	<i>Sphaerotheca macularis</i> , <i>Leveillula taurica</i>
Charcoal Rot	<i>Macrophomina phaseolina</i>
Olive Leaf Spot	<i>Pseudocercospora cannabina</i> , <i>Cercospora cannabis</i>
Brown Blight	<i>Alternaria alternata</i> , <i>A. solani</i> , <i>A. cheiranthi</i> , <i>A. longipes</i>
<i>Stemphylium</i> Leaf & Stem Spot	<i>Stemphylium botryosum</i> , <i>S. herbarum</i>
Southern Blight	<i>Sclerotium rolfsii</i>
Black Mildew	<i>Sciffnerula cannabis</i>
Twig Blight	<i>Dendrophoma marconii</i> , <i>Botryoshaeria marconii</i> ,
Pink Rot	<i>Trichothecium roseum</i>
<i>Cladosporium</i> Stem Canker	<i>Cladosporium herbarum</i> , <i>C. cladosporioides</i> , <i>C. enuissimum</i> , <i>C. resinae</i>
Anthraxnose	<i>Colletotrichum coccodes</i> , <i>C. dematium</i>
<i>Verticillium</i> Wilt	<i>Verticillium dahliae</i> , <i>V. albo-atrum</i>
Rust	<i>Aecidium cannabis</i> , <i>Uredo kriegneriana</i> , <i>Uromyces inconspicuus</i>
Black Dot	<i>Epicoccum nigrum</i>
Basidio Rot	<i>Athelia arachnoidea</i> , <i>A. epiphylla</i>
Red Boot	<i>Melanospora cannabis</i>
Texas Root Rot	<i>Phymatrichopsis omnivore</i>
<i>Ophiobolus</i> Stem Canker	<i>Ophiobolus cannabinus</i> , <i>O. anguillidus</i>
<i>Chaetomium</i> Diseases	<i>Chaetomium succineum</i> , <i>C. globosum</i> , <i>C. elatum</i> , <i>C. murorum</i>
<i>Phomopsis</i> Stem Canker	<i>Phomopsis arctii</i>
White Leaf Spot	<i>Phomopsis ganjae</i>
Pepper Spot	<i>Leptosphaerulina trifolii</i>
<i>Curvularia</i> Blight	<i>Curvularia cymbopognis</i> , <i>C. lunata</i>
<b>Kingdom: Chromista</b>	
Damping off	<i>Pythium aphanidermatum</i> , <i>P. ultimum</i> , <i>P. debaryanum</i>
Downy mildew	<i>Pseudoperonospora cannabina</i> , <i>P. cubensis</i>

**Damping off** can be caused by the species *Pythium aphanidermatum*, *P. ultimum* and *P. debaryanum*. These pathogens belong to the kingdom Chromista, type Oomycota from the Pythiaceae family (Serzane, 1962). They have been reported as the causal organisms of damping off in hemp in addition to other fungi such as *Botrytis cinerea*, *Macrophomina phaseolina*, *Rhizoctonia solani* and several *Fusarium* species e.g. *F. solani*, *F. oxysporum*, *F. sulphureum*, *F. avenaceum* and *F. graminearum* (Pietkiewicz, 1958; Bush, 1985; Patschk *et al.*, 1997). *Pythium* species produce two kinds of spores: motile zoospores with flagella that swim and spread from plant to plant and oospores which are produced sexually, lack flagella and allow the disease to persist as overwintering spores. The pathogen can attack the plant in two different scenarios: pre-emergence and post-emergence. In the former, spores inhabit the seed – overwintering and then limiting the germination rate. In the latter the seedlings and stems are affected, which become brown at the soil line and wilt (Kirchner, 1966).

**Viral diseases on *Cannabis sativa* L.** commonly cited in Europe are Hemp streak virus (HSV), Hemp mosaic virus (HMV), Alfalfa mosaic virus (AMV), Cucumber mosaic virus (CMV) and the Arabis mosaic virus (ArMV) (McPartland, 1996). HSV symptoms appears as a pale green chlorosis on leaves and it has been frequently reported in fibre hemp in Europe, while Hemp mosaic virus symptoms appear as a grey leaf mosaic – it has been reported on cultivars with high THC content. Similarly, to the other viruses, they are transmitted by insect vectors (Hartowicz *et al.*, 1971). Many insect species have been reported as virus-transmitters to hemp, such as Bhang aphids (*Phorodon cannabis*), greenhouse whiteflies (*Trialeurodes vaporariorum*), onion thrips (*Thrips tabaci*) and green peach aphids (*Myzus persicae*) (McPartland, 1996).

**Branch blight (broomrape)** caused by *Orobanche ramosa*, has been introduced to Central Europe and Poland since the beginning of the hemp cultivation in the 1850s (Piwowarczyk, 2012). The localization of *O. ramosa* in Poland is limited to few scattered locations in the lowland of Pomerania, Kujawy, Greater Poland, Sandomierz and Silesia, the Malopolska and Lubelska Uplands, as well as the Subcarpathian Foothills (Mađalski, 1967; Zajac & Zajac, 2001). The authors also listed Podolia and Volhynia in Ukraine, located nearby, as important regions of the presence of this parasite. The preferred hosts for *O. ramosa* are *Cannabis sativa*, *Nicotiana tabacum*, *Lycopersicon esculentum* and *Solanum tuberosum* (Piwowarczyk, 2012). Flowering time of this parasitic plant starts from March in southern Europe and from late August until late October in Poland. A single flower of this species produces between 700-4000 seeds, so every plant can produce 35000-500000 durable seeds leading to dramatic increase in the *Orobanche* seed bank in the soil (Kreutz, 1995). A few studies have been conducted on species suitable for biocontrol of broomrape, such as the insect with phytophagous larvae *Phytomyza orobanchiae* which was reported as feeding on different species of *Orobanche* (Klein & Kroschel, 2002).

## Pests in hemp cultivation

Pest occurrence in cannabis cultivation causes a threat to plant growth as well as the magnitude and quality of crop yield (Table 2). The first group, impacting growth rate and plant condition, are polyphagous soil pests. Depending on environmental and soil conditions, these represent different Lepidoptera species from the family Noctuidae, whose caterpillars damage plant root systems. The beetle larvae of the family Elateridae and Scarabaeidae forage

in a similar way. The threat they pose, however, can be minimized in the early stages of soil preparation for cultivation (Bunalski *et al.*, 2010).

Table 2. The pests attacking hemp in Poland

<b>Pest common name</b>	<b>Casual Organism</b>
<b>Nematodes - Tylenchida</b>	
Stem nematode	<i>Ditylenchus dipsaci</i>
<b>Spider mites – Acari</b>	
Carmine Spider Mite	<i>Tetranychus cinnabarinus</i>
Two-Spotted Spider Mites	<i>Tetranychus urticae</i>
<b>Bugs – Hemiptera</b>	
Black Bean Aphid	<i>Aphis fabae</i>
Green Peach Aphid	<i>Myzus persicae</i>
Hops Aphid	<i>Phorodon humuli</i>
Greenhouse Whitefly	<i>Trialeurodes vaporariorum</i>
Alfalfa Plant Bug	<i>Adelphocoris lineolatus</i>
Tarnished Plant Bug	<i>Lygus rugulipennis</i>
<b>Thrips – Thysanoptera</b>	
Onion Thrips	<i>Thrips tabaci</i>
Flower Thrips	<i>Frankliniella schultzei</i>
<b>Beetles – Coleoptera</b>	
Lined Click Beetle	<i>Agriotes lineatus</i>
May Bug	<i>Melolontha melolontha</i>
Garden Chafer	<i>Phyllopertha horticola</i>
Hemp Flea Beetle	<i>Psylliodes attenuata</i>
<b>Butterflies – Lepidoptera</b>	
European Corn Borers	<i>Ostrinia nubilalis</i>
Silver Y-Moth	<i>Autographa gamma</i>
Dot Moth	<i>Melanchra persicariae</i>
Cabbage Moth	<i>Mamestra brassicae</i>
Common Cutworms	<i>Agrotis segetum</i>
<b>Flies – Diptera</b>	
Crane Flies	<i>Tiplula paludosa</i>
Leaf Miners	<i>Agromyza reptans</i>
	<i>Chromatomyia horticola</i>
	<i>Liriomyza strigata</i>
Nettle Midge	<i>Melanogromyza urticivora</i>
Bean Seed Maggot	<i>Hylemyia florilega</i>
Seedcorn Maggot	<i>Delia platura</i>
<b>Birds – Aves</b>	
Hemp Linnet	<i>Carduelis cannabina</i>
Magpie	<i>Pica pica</i>
Starling	<i>Sturnus vulgaris</i>

A more serious issue is usually caused by pests foraging on above-ground plant parts. The diversity of species in this category is high which require additional descriptions. In the natural-climatic conditions of Poland, the most dangerous pests in hemp cultivation are: Damson-hop aphid (*Phorodon humuli*), European hop flea beetle (*Psylliodes attenuata*), European corn borer (*Ostrinia nubilalis*), and Silver Y moth (*Autographa gamma*). Damson-hop aphid (*Phorodon humuli*) is a small heteroecious aphid (1.8-2.1 mm in length). Out of their eggs, typically overwintering on plum trees, wingless fundatrices are hatched, which give birth to a winged generation of emigrants foraging on summer hosts, including cannabis plants. Subsequent larvae generations attack sprout tips and young leaves, foraging on their lower parts. At a later stage, they also move to inflorescences. They extract sap from vascular rings, and disrupt growth and cell division, thus weakening plants, deforming leaves and stems, and decreasing seed yield (Kochman & Węgorok, 1978).

The European hop flea beetle (*Psylliodes attenuata*) is a small beetle (1.5-2.5 mm) from the family Chrysomelidae. It forages on different plants from the family Urticaceae, including hemp. These beetles make holes in leaf blades what causes extensive damage to leaves if they are numerous. This leads to disruptions in photosynthesis, which, in turn, weakens growth, this may be especially damaging to young plants (Kochman & Węgorok, 1978).

The European corn borer (*Ostrinia nubilalis*) is a Lepidoptera belonging to the family Pyralidae. It does not pose a great threat to the plants of hemp. However, its caterpillars, despite having a preference for corn, can forage on other plants as well, including hemp. By gnawing sprouts, they cause them to wither and break, which can significantly impact the yield and quality of crops (Wilkaniec, 2012).

Silver Y moth (*Autographa gamma*) is an average-size Lepidoptera, which belongs to the family Noctuidae. Similarly, to the previous species, its caterpillars pose a threat to plants. The larvae of the Silver Y moth are polyphagous folivores, feeding on leaves of different plant species, including cannabis. When occurring in large numbers, the foraging of caterpillars leads to wide scale damage of leaves, and hence the reduction of the assimilative apparatus of plants. This leads to a decrease in crop yield and sometimes even its failure as a result of withering of plants (Bunalski *et al.*, 2010).

Apart from the discussed pests, the literature also details other pests foraging on cannabis such as the stem nematode (*Ditylenchus dipsaci*) and birds. Because of its feeding preferences, *Ditylenchus dipsaci* and its threat to cannabis should be confirmed by separate observations (Brzeski, 1993). There are no such doubts, unfortunately, when it comes to birds. Seeds constitute a perfect food for birds and many Passeriformes species take advantage of it. In case of small plantations, birds can decrease seed yield, impacting significantly crop profitability. European birds including hemp linnet (*Carduelis cannabina*), magpie (*Pica pica*) and starling (*Sturnus vulgaris*) were reported as prodigious seed eaters (Sorauer, 1958). Treating the seed with anthraquinone can be used as a bird repellent (Karus, 1997).

## Protection and management of hemp growth

The annually estimated loss in fibre of hemp reported by Agrios (1997) was 13% due to insects, 11% due to diseases and 7% due to weeds and other organisms. In addition to this, 9% losses occur during the postharvest phase (Pimentel *et al.*, 1991). The data does not include the damage caused by insects, where insects cause injury by feeding on the plant but they are transient factor not a continued casual factor (McPartland, 1994). These losses cause a substantial reduction in the profits of farmers, besides the cost of using chemical control (pesticides, fungicides and herbicides), which, moreover, increase the pollution of soils and

waters. More importantly, edible and medicinal products originating from hemp must be healthy and free from pesticides.

Nontoxic pest control is the keystone for sustainable agriculture. The introduction of crops to new growing areas will potentially expose them to attack by novel pests and unfamiliar diseases. Resistance against pests and diseases can be broken because the pathogens and pests often mutate and the population may change between the consecutive seasons. Pests can adapt within a short time from the introduction of new resistant cultivars (Gould, 1991). Breeding cultivars with significant resistance requires using large gene pools, different cultivars or different landraces from different countries or environments. Such an example may be a cross between dwarf northern Russian hemp and giant Southern Chinese hemp.

Diseases can spread between plants and settle very rapidly. One of the most useful solutions is an early detection system for the most damaging species. Maintaining the vigor of plants in a healthy condition and preventing any disease infections is a difficult task. The first stage of any strategy is to prevent the infection by choosing the best growing area with good airflow, low humidity and by avoiding overcrowding of plants in the field and avoiding areas with excessive water. Maintaining hygiene can involve regular observation of the plants during all the development stages, examining leaves especially the undersides where aphids and mites often congregate and by removing the residues of any infected plants. It is also necessary to check the stem around the base of the plant for cutworms and soil predators (Glick, 2012). Fungicide sprays should be used only in an emergency, as they can affect the bud quality by changing the taste, aroma and quality of the products. Plants used for pharmaceutical purposes can be sprayed with Neem oil or potassium soap to prevent plants from infestation, but this method is recommended on small fields only.

The presented overview of diseases and pests shows numerous natural enemies of hemp. Some of their pathogens are omnivorous, the others attack a group of plant families, whereas some are very specialized and they can be found mainly or solely on hemp. There were no detailed studies in this respect, but most presumably fibre- and oil-type hemp plants share the same health problems. By now, the cultivation area of hemp is small what prevents the pests and diseases from uncontrolled propagation. However, the cultivation of hemp becomes more and more popular, and the careful examination and monitoring of the situation is in demand.

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## **Ecologically-based Integrated Pest Management in oilseed rape: a need not an option**

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**Abstract:** Use of neonicotinoid seed treatments against insect pests in arable crops has been banned by the EU. There is widespread resistance of pest populations to the insecticides such as pyrethroids that can be sprayed onto crops. Farmers are running out of options for pest management.

Ecologically-based Integrated Pest Management is now a need – not an option. Integrated Pest Management is based on the setting of action thresholds, monitoring/risk assessment, prevention and control. It has been around for decades but examples of strategies used in practice in arable systems are few and far between. I will illustrate the development of an ecologically-based IPM strategy using examples from my team's work at Rothamsted on understanding the behavioural ecology of insect pests of oilseed rape – and that of their natural enemies. Action thresholds for most pests of oilseed rape are in place, but I will describe why these are not accurate and argue that dynamic thresholds are needed. By understanding insect immigration and host location processes, we have improved monitoring and risk assessment tools... but I believe the future lies with whole-field real-time detection and I will present some recent research on how we are exploring this possibility. In the absence of pest resistant cultivars, preventative methods which exploit our understanding of host-location processes are starting to be taken seriously by farmers and I will share my sweet-smelling and colourful vision of this future. Lastly, I will highlight our research into alternatives to synthetic toxicant insecticides, detailing advances in conservation biocontrol methods. Used together, these IPM tools will help to improve the agronomic, economic and environmental sustainability of this important crop.

**Key words:** IPM, insect pests, thresholds, monitoring, decision support systems (DSS), automated detection, LIDAR technology, insect resistant cultivars, conservation biocontrol

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## Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana* – the role of UV-protection in open field application

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### Background

Pollen beetles (*Brassicogethes* spp., previously named *Meligethes* spp., Coleoptera: Nitidulidae) are a major insect pest in oilseed rape and can cause substantial yield loss when present in large numbers. Their increasing resistance to pyrethroids coupled with deficient pest control possibilities in organic oilseed rape cultivation reinforces the demand for alternative control strategies to reduce pollen beetle infestation.

In previous studies, *Beauveria bassiana* showed high efficacy against pollen beetles in laboratory tests, but not in field experiments (Kuske *et al.*, 2013). Solar radiation is assumed to be one of the main factors that limit the efficacy of entomopathogenic fungi in open field applications (Ignoffo, 1992). Therefore, we tested various natural substances for their UV-protective potential of UV-B exposed *B. bassiana* conidia. We found humic acid as a promising novel UV-protective compound. In laboratory experiments, conidia formulated with 10% humic acid showed a survival rate of 100% in comparison to a survival rate of 22% in unformulated conidia. In a field trial in 2017, humic acid formulated conidia applied to oilseed rape buds showed significantly higher survival until day 7 after application.

In two field trials this spring (2018), we tested whether the addition of UV-protection to *B. bassiana* conidia influences their effect on pollen beetle control. To avoid an intermixture of pollen beetles between variants, we used large plots and in addition tents with a defined number of beetles. Survival of *B. bassiana* conidia and pollen beetle abundance was evaluated up to 14 days after application and the yield will be assessed following harvest in July.

### Material and methods

#### *Beauveria bassiana* conidia production and formulations

*Beauveria bassiana* strain ART2587 originates from a mycosed pollen beetle (*Meligethes* sp.) collected in Zurich, Switzerland (Pilz, 2006). *Beauveria bassiana* conidia were produced on sterile barley kernels by Mycelia GmbH, Nevele, Belgium, and harvested with a Mycoharvester (MH5, VBS Agriculture Ltd, Cornwall, UK). The oilseed rape field was divided into plots of 600 m<sup>2</sup> (30 m × 20 m) with 5 replicates per treatment. Test substances included: (1) 5 × 10<sup>13</sup>/ha *B. bassiana* conidia in 2% of the surfactant Telmion (Omya AG, Oftringen, Switzerland); (2) 5 × 10<sup>13</sup>/ha *B. bassiana* conidia in 2% Telmion and 5% humic acid (WH Pharmawerk Weinböhla GmbH, Weinböhla, Germany); (3) 5% humic acid in water and (4) Pyrinex (Chlorpyrifos, Syngenta, Dielsdorf, Switzerland).

### **Survival of *B. bassiana* conidia on oilseed rape buds**

At each sampling date (0, 3, 7 and 14 days after application) the main inflorescences of five randomly selected oilseed rape plants were collected from the center of each plot. In the laboratory, buds were homogenized with an electronic homogenizer (Bioreba, Reinach, Switzerland) and plated in triplicate on Sabouraud 2% Glucose Agar (SDA) containing antibiotics (Cycloheximide: 0.05 g/l, Streptomycinsulfate: 0.6 g/l, Tetracycline: 0.05 g/l) and the fungicide Dodine (50 mg/l). To get the average number of colony forming units (CFUs) per main inflorescence, the CFUs per total plant homogenate was calculated and divided by five.

### **Effect of application on pollen beetle abundance**

The abundance of pollen beetles was assessed before treatment and on subsequent days until 14 days after application. In each plot, 50 main inflorescences were collected and transferred to the laboratory for pollen beetle counting.

## **Preliminary results**

First results indicate no effect of the UV protectant humic acid on spore survival on assessed days after treatment. *Beauveria bassiana* spores formulated with humic acid caused the strongest decrease in pollen beetle abundance one day after treatment with a Henderson Tilton corrected efficacy of 21% , in comparison to an efficacy of 11% with unprotected spores and no effect of the humic acid alone or the insecticide. On the following days, no difference was seen on pollen beetle reduction between applied spores with and without humic acid.

## **Discussion**

Obtained results indicate the potential of *B. bassiana* to control pollen beetles but illustrate the lack of efficacy in field applications. As no effect of the UV-protectant humic acid was seen on spore survival, no clear statement can be drawn regarding the importance and effect of increased spore survival on pollen beetle control.

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## **Quantitative resistance to clubroot and interactions with abiotic stresses**

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**Key words:** *Plasmodiophora brassicae*, *Brassica napus*, *Arabidopsis thaliana*, abiotic/biotic stress crosstalk, disease resistance

**Extended abstract:** Quantitative resistance is considered today as a key lever to increase the durability of plant resistance against rapidly evolving pathogen populations (Brun *et al.*, 2010; Tomita *et al.*, 2013; Pilet-Nayel *et al.*, 2017). Introgression and managing of quantitative resistance is however difficult because it is conditioned by several genes having small effects on the phenotype and is also greatly influenced by the environment. An understanding of the mechanisms involved in the modulation of resistance QTL by abiotic parameters can help to rationalize the identification, combination and management of different genetic resistance factors.

Nitrogen fertilization and soil water content have been previously reported to affect the development of clubroot, a worldwide disease of the Brassicaceae caused by the soil-borne protist *Plasmodiophora brassicae*. In our lab, quantitative resistance to *P. brassicae* has been identified in *Brassica napus* (Manzanares-Dauleux *et al.*, 2000; 2006), in *B. oleracea* (Rocherieux *et al.*, 2004) and *Arabidopsis thaliana* (Jubault *et al.*, 2008). Our objective was then to assess the variability of clubroot resistance under nitrogen deficiency or in different climatic conditions (waterlogging, temperature) and to characterize the impact of these abiotic constraints on the effect of genetic factors involved in quantitative partial resistance to clubroot.

We developed combined genetic and molecular physiology approaches to investigate the influence of nitrogen fertilization on quantitative resistance of *B. napus* to clubroot. Disease response was studied in a panel of oilseed rape genotypes and *P. brassicae* isolates cultivated under low vs high nitrogen supplies. This work highlighted that lower nitrogen input can modulate disease symptoms (from strong symptom inhibition to no effect), depending on both the plant genotype and the *P. brassicae* isolate. QTL analysis conducted in a ‘Darmor-bzh’ x ‘Yudal’ doubled haploid progeny showed that nitrogen deficiency exerts a major switch between the effects of two QTL involved in resistance toward eH isolate (Laperche *et al.*, 2017). One low-nitrogen-dependent QTL identified on the chromosome C02 was found to exert a major effect on the resting spore content in infected roots and to moderately influence club symptom development. By contrast, the effect of a major QTL involved in resistance toward K92-16 isolate was unaffected by nitrogen fertilization. Altogether, our results indicated that nitrogen fertilization influence clubroot disease in a QTL x isolate dependent manner. A better understanding of QTL x pathogen isolate x fertilization crosstalk may help to rationalize the use of clubroot quantitative resistance in breeding.

In *A. thaliana*, waterlogging constraint applied during the secondary phase of infection modulated the effect of partial resistance, positively or negatively according to the QTL. These results suggest the existence of complex crosstalk between plant hypoxia response and plant response to clubroot (Gravot *et al.*, 2016). Temperature during infection process was also shown to modulate the level of club symptoms and the expression of quantitative resistance factors (Liégard *et al.*, submitted).

These results illustrate the importance of linking genetics and physiology approaches for the study of abiotic-biotic stress interactions and predict the modulation of resistance in various environments.

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**Entomology Session 1:  
Insects in oilseeds  
and alternative control options**



## Are rapeseed varieties resistant to *Turnip yellows virus* (TuYV) the solution against viruses transmitted by aphids?

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**Abstract:** Three viral species (i.e. *Turnip yellows virus* (TuYV), *Turnip mosaic virus* (TuMV) and *Cauliflower mosaic virus* (CaMV)) were detected in winter oilseed rape crops in France in the early 1990's. These viruses are transmitted by several aphid species including *Myzus persicae* which is reported to be present at a rate up to 60% on average in rapeseed fields in production areas in France. TuYV is the most prevalent virus reported so far in oilseed rape. Neonicotinoids (foliar spraying) is the last efficient insecticide family against *Myzus persicae* that can be used in rapeseed in autumn in France to protect this crop against vector-borne viral diseases. With the ban of this chemical family by the French authorities scheduled in September 2018, oilseed rape varieties resistant to viruses are of great interest. The first TuYV-resistant oilseed rape variety (cv. Architect) was registered in France in 2016. Trials carried out with different varieties including cv. Architect showed that infection rate and viral accumulation in infected plants were lower in the resistant varieties than in the susceptible control cv. DK Exception. The partial resistant phenotype of these varieties was associated to a yield gain when trials were under high viral pressure, while in trials with low virus pressure no yield gain was associated with the TuYV-resistance. Unfortunately, varieties resistant to TuYV are not resistant to the other two viruses (CaMV and TYMV) reported in oilseed rape crops which can occasionally strongly affect rapeseed production.

**Key words:** oilseed rape, *Myzus persicae*, viral diseases, *Turnip yellows virus* (TuYV), insecticide, yield

### Introduction

*Turnip yellows virus* (TuYV, *Polerovirus* genus, Luteoviridae family), a virus which induces yellowing symptoms on oilseed rape (*Brassica napus*) (Shattuck, 1992), is exclusively transmitted by the aphid *Myzus persicae* in a persistent manner (Brault *et al.*, 2010). This aphid species is commonly found in fields in all oilseed rape growing regions. National surveys of rapeseed crops highlighted that TuYV is the most important virus infecting this crop in autumn in France (Ballanger, 1997; 2002; 2009). Trials, carried out since 2010, have shown that an average yield loss of 0.25 t/ha can be associated to the presence of TuYV (Ruck, 2017). However, severe TuYV infections can lead to a yield loss of up to 1 t/ha as reported by Champagne-Cereales in 2010 (Ballanger, 2011).



Foliar spray of neonicotinoids is the last-remaining insecticide treatment available against *Myzus persicae* that can be used in rapeseed in autumn in France to protect this crop against vector-borne viral diseases (Ballanger, 2002; 2009; 2011; Fontaine, 2011; Ruck, 2017). However, for the next growing season, i.e. autumn 2018, it will PROBABLY not be possible to use an insecticide from this chemical family in France due to potential additional restrictions. Thus, characterization of TuYV-resistant rapeseed varieties is one of the most important challenges for the future of rapeseed production (Lüders, 2017). During the last decade, several TuYV-resistant rapeseed varieties (Architect (2016) and Angelico (2017) [Advanta], Temptation (2017) and Delice (2017) [DSV]) were registered in France. Allison (2015 [Advanta]), Coogan (2016 [RAGT]) and Smaragd (2018 [DSV]) were registered in the European seed catalog.

Despite the existence of several TuYV-resistant varieties since 2015 in France, little information on their behavior against TuYV infection is available. With the restrictions on the use of insecticides of the neonicotinoids family, the future wide deployment of these varieties requires a better characterization of the resistance phenotype.

## Material and methods

In 2016/17, six field experiments (one field per location) were conducted in the Grand Est region near Reims (Marne, France). The trial design included a TuYV-resistant variety (cv. Architect) and a TuYV-susceptible reference (cv. DK Exception). Plants were protected (Proteus 0.625 l/ha; a. i. Thiocloprid & Deltamethrin) or remained untreated (control) against aphids. Each trial was divided into 12 or 16 plots in a randomized block design with three or four replicates of each treatment. Insecticide sprays were applied when the French threshold of 20% of plants infested by aphids was monitored on cv. DK Exception (Table 1).

Table 1. Locations of field trials and insecticide treatments, 2016/17

Partner	Trial location (department)	Number of insecticide applications
Acolyance	Verneuil/Serre (02), Epoye (51)	1
Vivescia	Dizy le Gros (02), Cernon (51), Montiers sur Saulx (55)	2
	Prusy (10)	0

To estimate the TuYV infection rate of each plot, 10 plants (one leaf per plant) were sampled before winter (weeks 48 to 50). Sampled plants were randomly selected within the plots. The sanitary status of each sample was determined using a serologically-based assay (ELISA test). The presence of TuYV was validated by spectrophotometric measurements (OD<sub>405</sub>) using a microtiter plate reader. The viral load of infected samples was estimated using a standard curve produced with serially diluted fractions of an infected control.

All statistical analysis were carried out using SAS for Windows 9.4. The influence of the different parameters on the TuYV infection rate and virus load in infected plants was modelled by the GLM function. If models showed significance, the Dunnett's test at a significance level of 5% was done. Yield differences were analysed using an ANOVA Test.

## Results

During the monitored period, the trial located at Prusy did not reach the 20% threshold for insecticide treatment. Thus, this site was removed from the data set. For the 5 remaining sites, infection rates and viral load of infected plants were analysed and compared to data from the susceptible reference cv. DK Exception.

In the absence of chemical protection, the TuYV-resistant variety Architect presented in the three types (low, standard and severe) of viral pressure i) a significant lower rate of infection (with a decrease of up to 90% under severe TuYV infection) and ii) a significant lower viral load (up to 15 times) compared with the sensitive reference DK Exception. In trials with a low viral pressure, cv. Architect and cv. DK Exception, with or without chemical treatment, present similar response to virus infection. With a standard viral pressure, the yield improvement due to insecticide treatment is not significant. For experimental sites with severe infections, yield variation between the 2 tested varieties is higher for untreated plots (0.21 t/ha) than for treated plots (0.02 t/ha). Moreover, insecticide treatment led to a higher yield gain for the susceptible cv. DK Exception (0.51 t/ha) than for TuYV-resistant cv. Architect (0.28 t/ha).

Table 2. Infection rates of TuYV and viral loads of infected oilseed rape plants under low, standard and severe infections.

	Low infection*				Standard infection*				Severe infection*			
	Architect**		DK Exception**		Architect		DK Exception		Architect		DK Exception	
	Control	Proteus	Control	Proteus	Control	Proteus	Control	Proteus	Control	Proteus	Control	Proteus
% of infected plants	2.5	0	22.5	17.5	9	7	84	80	10	12.5	100	82.5
	b	b	a	a	b	b	a	a	c	c	a	b
Average viral load of plots	0.1	0	3.2	1.8	0.43	0.25	10.4	7	0.1	0.7	21.5	8.1
	b	b	a	ab	c	c	a	b	c	c	a	b
Viral load of infected plants	0.8	0	10.8	9.7	2.6	0.4	12.2	9.2	1.4	6.2	21.5	9.7
	b	b	a	a	b	b	a	a	c	bc	a	b
Yield (t/ha)	4.4	4.5	4.5	4.4	4.7	4.8	4.4	4.5	5.7	5.9	5.5	6
	a	a	a	a	ab	a	c	bc	ab	a	b	a

\*: the five trials were submitted to natural low (1 trial), standard (3 trials) or severe (1 trial) TuYV infections. Different letters indicate significant differences between varieties (Dunnnett's test ( $P \leq 5\%$ ))

\*\* : cv. Architect and cv. DK Exception are resistant and susceptible to TuYV infection, respectively. Untreated (control) and treated (insecticide treatment: Proteus) plots were monitored.

## Discussion

TuYV is the most important virus of oilseed rape in France. This virus mostly infects plants in autumn when its vector, the aphid *Myzus persicae*, reaches recently sown rapeseed crops. *Myzus persicae* is frequently observed in fields as reported by national surveys. This wide presence of the TuYV vector in crops facilitates emergence and spread of TuYV. In trials carried out since 2010, yield loss due to TuYV was estimated to be on average 0.25 t/ha. However, higher yield losses can be observed in severe infections as reported by Champagne-Cereales in 2010 (Ballanger, 2011) where two insecticide treatments allowed yield increase of 28%.

In 2016-17, field experiments were carried out to evaluate the TuVY-resistant phenotype of cv. Architect. Infection rates and virus load were compared to a susceptible reference (cv. DK Exception). In 5 trials, cv. Architect presented both a lower infection rate and a lower viral load in infected plants highlighting its partial resistant status. Moreover, the experiment allows testing cv. Architect under different virus pressures from low to severe infections. These contrasted situations made it possible to conclude that, except under low virus pressure, the TuVY-resistance of cv. Architect leads to yield gain. However, in trials with high virus pressure chemical treatments can also increase yields (especially for the susceptible reference). Insecticide treatments under low or standard virus pressures were not associated with significant variation in yield. This suggests that protecting rapeseed with insecticides was not always required even when the virus is present.

In the 5 trials, yield gain related to insecticide treatments applied to cv. Architect was never significantly higher than the yield from untreated plots. For the susceptible reference under severe infection, insecticide treatment increased the yield by 0.51 t/ha. The net gain (yield cost/benefit: rapeseed average sale price: 330 €/t, insecticide: 27 €/ha [chemical (17 €/ha) and spray (10 €/ha)] associated to the yield improvement due to insecticide treatment (2 sprays applied under severe infection) reached 116 €/ha for the reference cv. DK Exception and 40 €/ha for cv. Architect (yield increased: 0.28 t/ha). Under low or standard virus pressures, no gain was associated with the use of chemical treatment for both varieties.

In field experiments, it is not so easy to distinguish the targeted organism (i. e. TuVY) from the other biotic and abiotic parameters that could impact the yield. For these trials, the two other viral diseases that cause yield decrease in oilseed rape production, i.e. the mosaics induced by *Cauliflower mosaic virus* and *Turnip mosaic virus*, were not found in tested samples. Thus, in the absence of other biotic and abiotic stresses, the yield variations monitored between the plots can be assigned to *Turnip yellows virus*.

While it is undeniable that the partial resistance of cv. Architect brings a real advantage against TuYV, insecticide treatment was required under severe infection to maintain high yields. As virus pressure cannot be determined at sowing (or during the early stages of rapeseed development), it is still difficult to decide whether or not to avoid the use of insecticide without risking yield decrease. The partial TuYV resistance provided by cv. Architect is a first step in a new way (insecticide-free) to control TuYV in rapeseed fields. However, caution should also be applied on the consequences of a wide-scale deployment of this genetic resource. Indeed, as with any partial virus resistance, emergence of TuVY variant(s) that overcome the resistance gene present in cv. Architect germplasm is possible. Thus, characterization of the durability of the TuYV resistance remains to be evaluated.

To complete experiments carried out in 2016-17, field experiments with other putative TuYV-resistant genotypes were sown in autumn 2017 and the data are currently analysed.

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## **Take a load from the environment: The potential to minimize insecticide use by selenium fertilization in winter oilseed rape**

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**Abstract:** With 1.2 Million ha, oilseed rape (OSR) is one of the most valuable arable crops in Germany as well as in Europe. The crop is attacked by several insect pests which reduce yield drastically and lead to frequent use of insecticides that increases selection for resistance in pest populations and affect beneficials like pollinators, parasitoids and predators. The goal of this research is to deter insect pests from winter OSR by using selenium (Se) enriched fertilizers so that the use of insecticides (e. g. pyrethroids, neonicotinoids) is not necessary. At the same time, this approach counteracts the general Se deficiency in soils of most European countries. Regarding biofortification of OSR, Se-enriched OSR residues can substitute Se applied with mineral fertilizers in the long term. Overall, the Se enrichment has the potential to ‘kill 4-5 birds with one stone’: deterring insect pests from OSR, reducing insecticide use, enrichment of Se deficient soils by incorporation of plant residues; the Se enriched oilseed rape cake can be used as forage for livestock to cover their selenium demands and finally, enhanced Se accumulation can increase resistance to drought stress. Selenium is a micronutrient that is essential for mammals and many other organisms. Soils in Germany and many European countries are regarded as Se-deficient. Thus, addition of Se by fertilization is appropriate in arable crops. In this regard, OSR plays a special role because of its intensive sulphur (s) metabolism (e. g. by glucosinolates). Sulphur can be replaced by selenium. The physical and chemical similarities of Se and S help to explain the intimate association between Se and S metabolism in plants. The presence of isologous Se and S compounds indicates these elements compete in biochemical processes that affect uptake, translocation and assimilation throughout plant development. Thus, it is easy to produce OSR plants with a considerable Se content. Although there are several indications Se accumulation in plants deters herbivorous pest insects from OSR, up-to-date Se-S driven metabolism has never been tested on *Brassica napus* L. and its pests. Field trials with different cultivars and different treatments regarding Se and S were established and the deterring effect of Se on pest insects in oilseed rape was assessed. The first results from these trials are presented.

**Key words:** Oilseed rape, insect pests, Selenium, Sulphur, Cysteine, deterrence

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## Does distance matter? An investigation into the effect of distance of the previous oilseed rape crops on abundance of pollen beetle parasitoids and parasitism

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**Abstract:** Oilseed rape is an important cruciferous crop that is grown for its oil content. The oil is used by the food, chemistry and a biofuel industry, the meal is used as animal feed. Due to the growing demand for renewable energy sources the growing area of oilseed rape has expanded. Oilseed rape plants are attacked by several insects that are specialised on cruciferous plants but the pollen beetle (*Brassicogethes aeneus* Fab. syn. *Meligethes aeneus* Fab.) is one of the most widespread. The pollen beetle is a small black beetle with a metallic blue-green sheen. Adults emerge from overwintering sites in early spring and feed on the pollen of wild plants from different families. After maturation feeding they omit the polyphagous phase and only use cruciferous plants for oviposition. Pollen beetle larvae feed on pollen, but the main yield decreasing damage is caused by adults that feed on the buds of oilseed rape. Pest management of oilseed rape is based on synthetic insecticides that are often applied routinely without regard of pest incidence. Insecticides kill not only the pest but also the beneficial arthropods, e. g. parasitoids, pollinators and predatory arthropods, that provide essential pest control and pollination service to farmers. Therefore the development of ecologically viable and economically profitable pest management systems is urgently needed. The aim of this study was to find out whether the distance of previous year's oilseed rape has an impact on the abundance of the key parasitoids of pollen beetles and the parasitism rate of pollen beetle larvae.

The study was carried out in 12 commercially grown winter oilseed rape fields in Tartu County in 2017. Six oilseed rape fields were situated closer than 500 m to last year's oilseed rape and six were more than 500 m away from last year's oilseed rape fields. To assess the abundance of adult beetles and key parasitoids, yellow water trays were used from the green bud growth stage of oilseed rape plants until the end of flowering (BBCH 57-70). To estimate the abundance of larvae and their parasitism rate, funnel traps were used from the full flowering stage of plants until the beginning of the pod stage (BBCH 65-70).

In general, the abundance of adult pollen beetles was extremely low in the study year, which influenced our results. The results of our study showed that the number of key pollen beetle parasitoids were significantly greater in fields closer than 500 m to the previous year's oilseed rape fields. The number of larvae dropping to the soil was greater in the fields that were more than 500 m away from the previous year's oilseed rape field, however, their parasitism rate was greater in fields that were situated closer than 500 m to previous year's oilseed rape fields.

Our study showed that previous year's oilseed rape fields acted as a source for pollen beetle parasitoids but it did not support the larval abundance of beetles in the field. Parasitoids of pollen beetle overwinter in the oilseed rape field and if farmers use direct or minimised drilling after the oilseed rape crop, parasitoids will survive and can contribute to the biocontrol service. However, as the study describes data from only one-year when the number of overwintered pollen beetles was very low, so it is not possible to draw strong conclusions.

**Key words:** pollen beetle, *Brassicogethes aeneus*, *Meligethes aeneus*, key parasitoids, parasitism rate





## Spatio-temporal associations between the distributions of insect pests and their parasitoids in winter oilseed rape crops

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**Abstract:** From the group of potentially important parasitoids of the pollen beetles (*Brassicogethes aeneus*), *Tersilochus heterocerus* adults were the most abundant in oilseed rape crops during 2016 and 2017. The second most common parasitoid species found was *Phradis morionellus*. *Tersilochus tripartitus*, the main parasitoid of cabbage stem flea beetles (*Psylliodes chrysocephala*) was recorded only during spring 2017 – it coincided with markedly higher levels of damage induced by *P. chrysocephala* larvae at the time. For *Ceutorhynchus obstrictus*, all three important pteromalids (*Stenomalina gracilis*, *Trichomalus perfectus*, and *Mesopolobus morys*) were recorded in similar abundances. From the known parasitoids of *Dasineura brassicae*, only *Omphale clypealis* was recorded in 2017. Significant spatial associations ( $p < 0.025$ ) between the distribution patterns of *B. aeneus* adults and *T. heterocerus* adults (both sexes assessed together in this case) were recorded on some dates. The parasitisation of pollen beetle larvae by *T. heterocerus* proved to be high in untreated crops: on average 56% and 87.5-100% larvae were parasitized in 2016 and 2017, respectively). This indicates that parasitoids should be effective biological control agents of *B. aeneus* populations.

**Key words:** winter oilseed rape, insect pests, parasitoids, *T. heterocerus*, *B. aeneus*, spatial distribution, spatial association

### Introduction

Winter oilseed rape is the most important oilseed crop for Czech farmers. During recent years winter oilseed rape has been grown on acreage of almost 400,000 hectares in the Czech Republic (Volf & Zeman, 2017). The hymenopteran parasitoids belonging to various families present a substantial and important part of the group of natural enemies of all insect pests usually present in the crop (Ulber *et al.*, 2010). Parasitoids can substantially influence the abundance of the oilseed rape insect pests in the crops from the long-term even short-term point of view (Šedivý, 1983). However, there are almost no data which could be used to help farmers to incorporate these organisms as important biological agents into practical pest management schedules in the Czech Republic. The aims of this study are to:

- Determine which parasitoid species play significant roles in influencing population dynamics of the main insect pests of oilseed rape;
- Determine spatio-temporal distributions of the most important insect pests and their parasitoids;
- Investigate any spatio-temporal associations between the distributions of pollen beetles and some of their main parasitoids (for purposes of this study we concentrate on *B. aeneus* and its main parasitoid *T. heterocerus*).

## Material and methods

In the course of 2016-2017 the spatio-temporal distributions of the main insect pests of winter oilseed rape *Brassicogethes aeneus* (Fabricius, 1775), *Ceutorhynchus obstrictus* (Marsham, 1802); *Ceutorhynchus pallidactylus* (Marsham, 1802); *Dasineura brassicae* (Winnertz, 1853); *Psylliodes chrysocephala* (Linnaeus, 1758) and their parasitoids (*Phradis morionellus* (Holmgren, 1860); *Tersilochus heterocerus* Thomson, 1889; *Tersilochus tripartitus* (Brischke, 1880); *Tersilochus obscurator* Aubert, 1959; *Blacus nigricornis* Haeselbarth, 1973; *Diospilus capito* (Nees, 1834); *Omphale clypealis* (Thomson, 1878); *Stenomalina gracilis* (Walker, 1834); *Mesopolobus morys* (Walker, 1848); *Trichomalus perfectus* (Walker, 1835) were assessed in oilseed rape crops. The monitoring areas were approximately 1 ha (100 × 100 m). In both seasons the crops were located near Šumperk (North-eastern area Czech Republic). We concentrated on adults of the species. For the purposes of their monitoring in-crop (more exactly for the monitoring of their flight activities) we used yellow water traps located in a grid pattern (5 × 5 = 25 traps) within the crop. The traps were emptied twice each week from the end of February to approximately the first half of June (to be sure we recorded the whole periods of the flight activities of the target species. The samples of insects from the individual traps and dates were preserved in ethanol until they were analysed in the laboratory. For every date and every trap the numbers of males and females of the above mentioned species were recorded.

For analysing spatial distributions of the individual species and their sexes *Spatial Analysis by Distance IndicEs* (SADIE) was used (Perry 1995; 1996). Aggregation indices  $I_a$  were calculated for males and females of all recorded species for each sampling date. In the case of non-random distribution with significant tendency of the assessed individuals to aggregate in clusters (patch or gap clusters), the value of  $I_a$  is higher than unity ( $I_a > 1$  for  $p < 0.05$ ). For the calculations SadieShell software version 2.0 was used (Perry 1995; 1996; 1998; see also Ferguson *et al.*, 2006).

To compare two distributions (in this study we concentrate on distributions of *B. aeneus* vs. *T. heterocerus* from various dates), SADIE-Quick Association calculates an overall spatial association index, X (upper case chi), based on the similarity of the local clustering indices from the two distributions – these were calculated earlier as described in the previous paragraph (Perry, 1998; Ferguson *et al.*, 2006). Values of X are  $> 0$  for distributions that are associated, around zero for distributions positioned at random with respect to one another, and  $< 0$  for distributions that are dissociated. The significance of X is tested by comparison with randomizations in which values of the local cluster indices are reassigned among the sample locations (Perry, 1998).

At the time of occurrence of *B. aeneus* larvae in crops they were repeatedly sampled and in the laboratory the frequencies of their parasitisation (induced by *Tersilochus* spp., *Phradis* spp.) were recorded.

## Results and discussion

From the group of potentially important parasitoids of pollen beetles, *T. heterocerus* adults were the most abundant species in both seasons. In both seasons males highly predominated to females in yellow water traps in the course of the whole flight periods. The second most common parasitoid was *P. morionellus*, which also showed also a high predominance of males in catches. The other known parasitoids of pollen beetles were substantially less abundant (*B. nigricornis*, *D. capito*) in yellow water traps (Table 1) or they were not record at

all (many other species is described in scientific papers; Ulber *et al.*, 2010). *Tersilochus tripartitus*, the main parasitoid of cabbage stem flea beetles (*P. chrysocephala*) was recorded only during the spring 2017; it coincided with markedly higher levels of damage induced by *P. chrysocephala* larvae at the time. For *C. obstrictus*, all three important pteromalids were recorded in similar abundances. From the known parasitoids of *D. brassicae*, only *Omphale clypealis* was recorded in 2017.

Table 1. Abundance of parasitoids of insect pests of oilseed rape recorded in yellow water traps located in winter oilseed rape crops in 2016 and 2017 (Šumperk, North East part of the Czech Republic) (total counts recorded during the whole periods of monitoring in the both seasons are presented).

Family of Hymenoptera	Species of parasitoids	2016	2017	Pest
Ichneumonidae	<i>Phradis morionelus</i> ♂	207	28	<i>B. aeneus</i>
Ichneumonidae	<i>Phradis morionelus</i> ♀	36	0	<i>B. aeneus</i>
Ichneumonidae	<i>Tersilochus heterocerus</i> ♂	3390	10248	<i>B. aeneus</i>
Ichneumonidae	<i>Tersilochus heterocerus</i> ♀	635	260	<i>B. aeneus</i>
Ichneumonidae	<i>Tersilochus tripartitus</i>	0	50	<i>P. chrysocephala</i>
Ichneumonidae	<i>Tersilochus obscurator</i> ♀	3	27	<i>C. pallidactylus</i>
Braconidae	<i>Blacus nigricornis</i> ♀	11	4	<i>B. aeneus</i>
Braconidae	<i>Diospilus capito</i>	0	16	<i>B. aeneus</i>
Eulophidae	<i>Omphale clypealis</i>	0	48	<i>D. brassicae</i>
Pteromalidae	<i>Stenomalina gracilis</i>	5	20	<i>C. obstrictus</i>
Pteromalidae	<i>Trichomalus perfectus</i>	3	20	<i>C. obstrictus</i>
Pteromalidae	<i>Mesopolobus morys</i>	1	20	<i>C. obstrictus</i>

The adults of all species and sexes (insect pests and their parasitoids) showed a strong tendency towards aggregation on some dates, especially at the time when they were highly active in crops (Perry, 1995; Hlavjenka *et al.*, 2016; Pearce & Zalucki, 2006).

Significant spatial associations ( $p < 0.025$ ) between the distribution patterns of *B. aeneus* adults and *T. heterocerus* adults (both sexes assessed together in this case) were recorded on some dates. This indicates the species should play an important role in diminishing pollen beetle populations during their development in crops, because it showed a relatively clear tendency (for some dates statistically significant) to concentrate in zones where higher pollen beetle abundance was recorded. These species were probably actively searching for the zones with high abundance of *B. aeneus* because at these places the probability of host larvae occurrence is higher (Tables 2 and 3).

Table 2. Spatial associations (or dissociations) between the distribution patterns of *Brassicogethes aeneus* adults and *Tersilochus heteroceris* adults in the course of the monitoring period in oilseed rape crops in 2016.

<i>B. aeneus</i>	<i>Tersilochus heteroceris</i>					<i>Tersilochus heteroceris</i>				
	Overall Spatial Index of Association					The levels of significance				
	<i>X</i>					<i>p</i>				
<i>Date</i>	<i>II.5</i>	<i>III.5</i>	<i>IV.5</i>	<i>I.6</i>	<i>II.6</i>	<i>II.5</i>	<i>III.5</i>	<i>IV.5</i>	<i>I.6</i>	<i>II.6</i>
11.4.	-0.210	-0.051	0.116	0.080	-0.031	0.843	0.585	0.2919	0.358	0.562
14.4.	-0.351	-0.137	0.2705	0.345	0.166	0.958	0.743	0.0987	0.063	0.209
18.4.	-0.206	-0.339	-0.4265	-0.024	0.216	0.779	0.911	0.9474	0.547	0.145
21.4.	0.215	-0.292	-0.1271	0.018	-0.239	0.199	0.923	0.7221	0.475	0.866
25.4.	-0.149	-0.329	-0.095	<b>0.428</b>	0.141	0.767	0.943	0.6788	<b>0.018*</b>	0.263
28.4.	-0.393	-0.384	-0.3868	0.230	0.048	0.972	0.972	0.9714	0.155	0.410
2.5.	-0.380	<b>-0.410</b>	-0.4061	0.342	0.161	0.971	<b>0.979**</b>	0.9706	0.054	0.229
5.5.	<b>-0.452</b>	<b>-0.401</b>	-0.2866	-0.395	-0.336	<b>0.990**</b>	<b>0.978**</b>	0.9204	0.958	0.954
9.5.	<b>-0.626</b>	<b>-0.504</b>	-0.0803	0.014	0.152	<b>0.999**</b>	<b>0.996**</b>	0.6398	0.475	0.231
13.5.	0.210	0.262	-0.0961	<b>-0.627</b>	-0.387	0.160	0.101	0.6694	<b>0.999**</b>	0.971
16.5.	0.243	0.277	-0.0929	-0.086	-0.315	0.123	0.087	0.6725	0.670	0.931
20.5.	-0.046	0.084	0.101	-0.320	-0.028	0.593	0.345	0.321	0.928	0.554
23.5.	0.070	0.208	0.1157	<b>-0.494</b>	<b>-0.420</b>	0.395	0.192	0.3308	<b>0.994**</b>	<b>0.982**</b>
26.5.	-0.097	0.146	0.1167	<b>-0.407</b>	-0.233	0.684	0.244	0.2838	<b>0.979**</b>	0.849
30.5.	<b>-0.502</b>	-0.178	-0.0982	-0.346	-0.098	0.994	0.809	0.6915	0.951	0.671
2.6.	-0.056	0.180	0.0355	-0.287	-0.204	0.608	0.195	0.4309	0.919	0.820
6.6.	<b>0.396</b>	0.371	0.2536	<b>0.537</b>	0.367	<b>0.025*</b>	0.034	0.1155	<b>0.003*</b>	0.042
9.6.	-0.081	0.021	-0.0328	0.351	<b>0.598</b>	0.648	0.454	0.5597	0.043	<b>0.003*</b>

\*\* significant association (when the values of *X* are positive and  $p < 0.025$ ) or significant dissociation (when the values of *X* are negative and  $p > 0.975$ ).

In some cases we recorded significant spatial dissociations between the distribution patterns of two parasitoids (*T. heteroceris* vs. *P. morrionelus*).

The parasitization of pollen beetle larvae induced by *T. heteroceris* proved to be high in the untreated crop: on average 56% in 2016 and 87.5-100% larvae parasitized in 2017. This indicates that the parasitoid species has the potential to be an efficient biological control agent of *B. aeneus* populations.

Table 3. Spatial associations (or dissociations) between the distribution patterns of *Brassicogethes aeneus* adults and *Tersilochus heteroceris* adults in the course of their presence in crops in 2017.

<i>B. aeneus</i>	<i>Tersilochus heteroceris</i>					<i>Tersilochus heteroceris</i>				
	Overall Spatial Index of Association					The levels of significance				
	<i>X</i>					<i>p</i>				
<i>Date</i>	<i>I.5</i>	<i>II.5</i>	<i>III.5</i>	<i>IV.5</i>	<i>I.6</i>	<i>I.5</i>	<i>II.5</i>	<i>III.5</i>	<i>IV.5</i>	<i>I.6</i>
30.3.	0.027	-0.138	-0.342	0.175	<b>0.531</b>	0.465	0.679	0.918	0.245	<b>0.018*</b>
3.4.	<b>0.629</b>	0.059	-0.490	-0.367	<b>0.596</b>	<b>0.006*</b>	0.401	0.984	0.933	<b>0.006*</b>
6.4.	<b>0.525</b>	0.141	-0.303	-0.253	<b>0.498</b>	<b>0.010*</b>	0.288	0.927	0.862	<b>0.021*</b>
10.4.	-0.338	0.123	-0.132	<b>0.499</b>	0.273	0.898	0.328	0.710	<b>0.016*</b>	0.167
13.4.	0.299	0.103	0.052	-0.292	<b>0.770</b>	0.137	0.339	0.414	0.875	<b>0.000*</b>
21.4.	0.022	-0.421	-0.215	0.382	0.131	0.461	0.945	0.817	0.052	0.315
25.4.	-0.394	0.017	0.049	<b>0.458</b>	<b>-0.504</b>	0.942	0.466	0.425	<b>0.025*</b>	<b>0.989**</b>
28.4.	<b>-0.432</b>	<b>-0.491</b>	-0.155	<b>0.504</b>	0.039	<b>0.975**</b>	<b>0.986**</b>	0.746	<b>0.010*</b>	0.426
1.5.	-0.119	<b>-0.663</b>	-0.297	0.007	0.326	0.670	<b>0.997**</b>	0.901	0.486	0.104
5.5.	-0.113	-0.296	-0.027	<b>0.469</b>	0.245	0.649	0.859	0.536	<b>0.024*</b>	0.182
9.5.	0.289	0.268	0.018	0.006	<b>0.490</b>	0.115	0.117	0.462	0.487	<b>0.012*</b>
15.5.	0.095	-0.060	0.270	-0.197	-0.157	0.352	0.585	0.116	0.796	0.720
22.5.	0.031	0.263	0.283	0.042	-0.479	0.444	0.182	0.094	0.433	0.975

\*\* significant association (when the values of *X* are positive and  $p < 0.025$ ) or significant dissociation (when the values of *X* are negative and  $p > 0.975$ ).

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## **Pests of stored oil crops**

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**Abstract:** Oil crops hold an important position in the economy of developed as well as developing countries as the sources for the edible oils and oils for industrial uses. Protecting stored oilseed crops from spoilage is an essential part of their production; failure to do so may result in them being downgraded. Storing oilseeds is more difficult than storing cereal grains, as they are more susceptible to quality deterioration and have limited insect control options. The decision to store oilseeds requires a planned approach, careful management and a suitable storage system. The rate of quality deterioration in stored oilseeds depends on the quality of grain placed in storage and management of temperature, moisture content and insects. Studies on storage and insect pest control in oilseeds are relatively few in comparison with those on cereal grains. Stored-product beetles often appear similar but have differing behaviour patterns and status as pests. It is important to determine which species are present before taking remedial action. The general recommended storage conditions for oil crops are: below 25 °C and below 7% moisture content, but vary according to the oil content. Oilseeds as well as oilcakes/meals are rich in proteins and fats and hence are vulnerable to infestation by stored product pests, resulting in weight loss, deterioration in quality and flavour, contamination, mould growth and formation of toxins. In these commodities, *Tribolium* spp. among beetle pests and *Ephestia cautella* as well as *Plodia interpunctella* among moths are important. These insects tend to favour the top of the grain stack and around silo outlets. Under optimum breeding conditions of about 30 °C, insects can complete their full life cycle in as little as four weeks. Reducing the grain temperature with aeration cooling plays a vital role in lengthening the insect breeding life cycle or in most cases stopping reproduction if cooled to below 18 °C.

The aim of this research was to determine, over a period of six months, the abundance of harmful oilseeds entomofauna in the storage facilities of experimental station Šašinovečki Lug. The Experimental station has an open type warehouse that allows better temperature regulation, airflow and ventilation in dry conditions. There is no temperature control in the storage facility, nor control of relative humidity. Stored oil crops were packed in one-toned bags made of polyester material. The oilseed grains were sampled using a special probe for grain sampling, which was "stacked" in a bag. The sampling was carried out from the bottom, the middle and the top of the sack, with a collective sample made of 1/3 of each layer sampled. The oilseeds (oilseed rape and turnip rape) were sampled once a month between 16 November 2017 and 15 April 2018. In total, both oil crops were sampled six times, each time taking four sub-samples (total of 48 analyzed sub-samples, each weighing 250 g). During the whole research period, physical properties (moisture, weight after sampling, weight after incubation and hectolitre mass) of oilseed rape and turnip rape seeds were analysed immediately after sampling, and the presence of entomofauna was observed. Each examined sample was then put into incubation for one month and the same procedure of analysis was performed (physical properties and visual inspection for insects). Entomofauna found after both examinations was identified to species level using standard determination keys.

The results of grain physical properties showed that, during the six month period, the oilseed rape grains had optimal average moisture (8-9%), while turnip rape average moisture was slightly lower (7-8%). In the first three samplings (November-January), the oilseed rape absolute weight increased by 10%, while the turnip rape weight increase was 20%, followed by weight loss in both oil crops until April. Overall, the oilseed rape absolute weight decreased by 12% and turnip rape by 15% when compared with the first sample. Weight loss was also detected after the incubation period, with 5% of maximum weight loss for oilseed rape in February and 12% for turnip rape in December. The hectolitre mass values were standard for both oil crops. In total, 152 insects were detected immediately after the sampling procedure and additionally, 25 specimens were detected after the incubation period. All 177 insects belonged to the order Coleoptera. Of these, 94% were found in turnip rape samples. In oilseed rape, species *Oryzaephilus surinamensis* (L.) was detected both before and after the incubation, and the only other species was *Cryptolestes ferrugineus* (Steph.), found after incubation in February (both in low numbers). The most abundant species in turnip rape detected during all six months was *Sitophilus granarius* (L.) (81%), followed by *Oryzaephilus surinamensis* (13%). Other species found in turnip rape were *Tribolium confusum* J. du Val (3%), *Gnathocerus cornutus* (F.) (1.3%), *Cryptolestes ferrugineus* (Steph.) (0.7%) and *Bruchus pisorum* (L.) (0.7%). After the incubation period, *Sitophilus granarius* and *Oryzaephilus surinamensis* were similarly abundant as immediately following sampling and were the only insects found.

Although the analyzed oil crops had optimal average moisture and values of hectolitre mass, the presence of insect pests presumably had an effect on stored grains weight loss, which in the end causes poor grain quality and yield loss. The most abundant detected species *Sitophilus granarius* is a primary pest found mostly on stored cereals. It is considered the most common polyphagous insect in storehouses and prefers higher grain moisture. However, we found it in highest numbers on turnip rape, which had somewhat lower moisture than oilseed rape. Presumably, some other factors, like optimal temperature, were influential in successful development of this species on turnip rape. Potentially, if these conditions change and become more favourable, we could expect to find this storage pest on oilseed rape, and also many other pests on different stored products. In order to ensure better storage conditions, it is necessary to disinfect the empty storage space, and upon receipt of the goods, continuously monitor the moisture and temperature of the products and the presence of harmful entomofauna, in order to react in a timely manner and thereby avoid greater losses.

**Key words:** stored products, damage, storage, harmful entomofauna, Coleoptera, Lepidoptera, oilseeds

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## Carabid beetle fauna of soybean in Croatia

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**Abstract:** The Carabid beetle fauna in soybean, *Glycine max* Merrill, was investigated in Croatia during 2016. Habitats sampled included two field sites in Croatia, Tovarnik in the east and Lukač in the west. Altogether, 35 species were recorded. Pitfall trapping indicated greater carabid abundance in western (Lukač), where eudominant species were *Pterostichus melas italicus* (Dejean, 1828), *Harpalus distinguendus* (Duftschmidt, 1812) and *Pterostichus melanarius melanarius* (Illiger, 1798). As euconstant, 11 species were identified. In the east (Tovarnik), eudominant species were *Harpalus rufipes* (DeGeer, 1774) and *Anchomenus dorsalis* (Pontoppidan, 1763), whereas *H. distinguendus* and *H. rufipes* were euconstant. On both sampling sites *H. rufipes* had the greatest ecological significance.

**Key words:** carabid fauna, Croatia, dominance, frequency, soybean

### Introduction

Soybean [*Glycine max* (L.) Merr.] is one of the most important commercial crops in cultivation. Seeds contain over 40% protein and 20% oil as well as other high value products such phospholipids, saponins, isoflavones, oligosaccharides and edible fibre (Subramanian and Smith, 2013). In Croatia, the average yield of soybean is around 3 t/ha and the acreage grown is over 50 000 ha with indication production increase over the last decade (Sudarić, 2013). According to Bažok *et al.* (2013) there are 13 harmful arthropod species reported as soybean pests in Croatia, although they are mostly not regularly controlled by insecticides in normal farming practice; control is necessary only in cases of high pest outbreaks. Soybean production is mostly carried out using integrated pest management where preservation of beneficial organisms is among the priorities. Carabid beetles are one of the most important beneficial organisms, known as predators of wide range of plant pests (Lövei and Sunderland, 1996). They are also one of the most abundant and diverse groups overwintering within cultivated fields (Pfiffner & Luka, 2000).

The aim of this study was to determine the carabid fauna in soybean crops in Croatia and to determine species abundance, dominance and frequency.

### Material and methods

The study was conducted in 2016 in Virovitica-Podravina County in Lukač (western Croatia) and Vukovar-Sirmium County in Tovarnik (eastern Croatia) which differ in climatic conditions. Ground beetles were collected four times during the cropping season, in May, July, August and September. Samples were collected using 40 epigeic covered pitfall traps per field. Traps were filled with saturated water-salt solution for carabid preservation. All specimens were determined to species by taxonomy expert Mr. Teun van Gijzen from the

Netherlands, following the keys of Auber (1965), Bechyne (1974) and Harde & Severa (1984). Nomenclature verification was carried out according to Vigna Taglianti (2013). According to Tischler (1949) and Heydemann (1955), species were classified as eudominant (> 10.00%), dominant (5.00-9.99%), subdominant (1.00-4.99%), recedent (0.50-0.99%) and subrecedent (0.01-0.49%). The frequency of species was calculated using the Balogh's formula (Balarin, 1974)  $C = nsA/Ns \times 100$ , where nsA represents the number of samples that contained species A and Ns the total number of samples. The results were classified according to Tischler (1949): euconstant (> 75%), constant (50-74.99%), accessory (25-49.99%) and accidental (< 24.99%). Based on these parameters, the ecological significance (W) was calculated. It is a synthetic index that represents the relationship between the structural index (C) and the productivity index (D) of the community ( $W = (CA \times DA) \times 100$ , CA – frequency of species A, DA – dominance of species A) (Varvara *et al.*, 2001). Data on weather conditions (mean weekly air temperature, mean weekly soil temperature and the total amount of rainfall) from 1<sup>st</sup> January-30<sup>th</sup> September were collected from the nearest Croatian Meteorological and Hydrological Service station, e. g. the station in Virovitica (VT) for Lukač and in Gradište (GR) for Tovarnik.

## Results and discussion

In total, 35 different species were determined as carabid fauna of soybean from the investigated fields in Croatia (Table 1). Although no carabid species appears to be strictly bound to a certain crop, early agro-ecological studies in Europe reported a general difference between ground beetle abundance distributions in winter versus spring crops (Heydemann, 1955).

In Lukač, western Croatia 1471 individuals were collected in total and classified into 28 species. Eudominant species were *Pterostichus melas* (Creutzer, 1799), *Harpalus distinguendus* (Duftschmid, 1812) and *Pterostichus melanarius* (Illiger, 1798). In May, *H. distinguendus* was more active when its abundance reached 325 individuals, while *P. melas* and *P. melanarius* were more active from August to September with numbers between 156 and 194 collected individuals (Table 2). According to Brigić *et al.* (2003), *P. melas* is dominant and common in Croatia in agricultural land near the Nature park Lonjsko polje. About 48% of species were accessory, followed by euconstant (40%) and constant (11%) species (Figure 1). There were no accidental species. Eudominant species in Lukač were also found to be euconstant. According to frequency, *H. distinguendus*, *Poecilus cupreus cupreus* (Linné, 1758), *Harpalus rufipes* (DeGeer, 1774), *P. melanarius*, *P. melas*, *Abax carinatus carinatus* (Duftschmid, 1812), *Anchomenus dorsalis* (Pontoppidan, 1763), *Anisodactylus signatus* (Panzer 1796), *Clivina fossor fossor* (Linnaeus, 1758), *Carabus cancellatus* (Illiger, 1798), and *Carabus granulatus* (Linnaeus, 1758) were classified as euconstant, species whereas *Calathus fuscipes* (Goeze, 1777), *Harpalus affinis* (Schrank, 1781) and *Stomis pumicatus* (Panzer, 1796) were constant (Figure 1).

Table 1. List of carabid beetle fauna collected in soybean crops in Lukač and Tovarnik, Croatia, 2016.

### **Carabid beetle fauna of soybean in Croatia**

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- Abax (Abacopercus) carinatus* subsp. *carinatus* (Duftschmid, 1812)  
*Agonum (Olisares) viridicupreum* (Goeze, 1777)  
*Amara (Amara) similata* (Gyllenhal, 1810)  
*Anchomenus (Anchomenus) dorsalis* (Pontoppidan, 1763)  
*Anisodactylus (Pseudanisodactylus) signatus* Panzer 1796  
*Bembidion (Metallina) properans* (Stephens 1828)  
*Bembidion (Bembidion) quadrimaculatum* (Linne, 1761)  
*Brachinus (Brachinus) crepitans* (Linnaeus, 1758)  
*Brachinus (Brachinus) elegans* Chaudoir, 1842  
*Calathus (Neocalathus) micropterus* (Duftschmid, 1812)  
*Calathus (Calathus) fuscipes* subsp. *fuscipes* (Goeze, 1777)  
*Callistus (Callistus) lunatus* subsp. *lunatus* (Fabricius, 1775)  
*Carabus (Carabus) granulatus* Linnaeus, 1758  
*Carabus (Procrustes) coriaceus* Linne, 1758  
*Carabus (Carabus) cancellatus* Illiger, 1798  
*Clivina (Clivina) fossor fossor* (Linnaeus, 1758)  
*Cylindera (Cylindera) germanica* (Linne, 1758)  
*Diachromus (Diachromus) germanus* (Linne, 1758)  
*Harpalus (Harpalus) affinis* (Schrank, 1781)  
*Harpalus (Harpalus) dimidiatus* (P. Rossi, 1790)  
*Harpalus (Harpalus) tardus* (Panzer, 1797)  
*Harpalus (Pseudoophonus) rufipes* DeGeer, 1774  
*Harpalus (Harpalus) distinguendus* Duftschmidt, 1812  
*Laemostenus (Pristonychus) terricola* (Herbst, 1784)  
*Nebria (Nebria) brevicollis* (Fabricius, 1792)  
*Parophonus (Parophonus) dejeani* (Csiki, 1932)  
*Poecilus (Poecilus) cupreus cupreus* Linné, 1758  
*Pseudoophonus (Platus) calceatus* (Duftschmid, 1812)  
*Pseudoophonus (Pseudoophonus) griseus* (Panzer 1797)  
*Pterostichus (Morphosoma) melanarius melanarius* Illiger, 1798  
*Pterostichus (Pterostichus) melas italicus* (Dejean, 1828)  
*Pterostichus (Pterostichus) niger niger* (Schaller, 1783)  
*Stomis (Stomis) pumicatus* (Panzer, 1796)  
*Trechus (Trechus) quadristriatus* (Schrank, 1781)  
*Zabrus (Zabrus) tenebrioides* (Goeze, 1777)

Table 2. Classification of species dominance and ecological significance of carabid beetles collected in soybean, Lukač, Croatia 2016.

<b>Species</b>	<b>*D (%)</b>	<b>Classification</b>	<b>W</b>
<i>P. melas</i>	24.47		24.47
<i>H. distinguendus</i>	23.79	Eudominant	23.79
<i>P. melanarius</i>	18.63		18.63
<i>H. rufipes</i>	7.68		76.80
<i>P. cupreus</i>	5.98	Dominant	59.80
<i>B. elegans</i>	5.71		14.28
<i>A. signatus</i>	4.08		30.60
<i>C. cancellatus</i>	2.65	Subdominant	19.88
<i>A. dorsalis</i>	2.18		16.35
<i>N. brevicollis</i>	1.02		2.55
<i>H. affinis</i>	0.75	Recedent	3.75
<i>C. granulatus</i>	0.54		4.05
<i>C. fuscipes</i>	0.41		2.05
<i>A. carinatus</i>	0.41		3.08
<i>C. coriaceus</i>	0.41		1.03
<i>C. fossor</i>	0.27		2.03
<i>B. quadrimaculatum</i>	0.14		1.05
<i>C. germanica</i>	0.14		3.40
<i>S. pumicatus</i>	0.10		6.80
<i>A. viridicupreum</i>	0.07	Subrecedent	1.70
<i>C. lunatus</i>	0.07		1.70
<i>P. dejeani</i>	0.07		1.70
<i>H. tardus</i>	0.07		1.70
<i>T. quadristriatus</i>	0.07		1.70
<i>P. niger</i>	0.07		1.70
<i>A. similata</i>	0.07		1.70
<i>B. properans</i>	0.07		1.70
<i>D. germanus</i>	0.07		1.70

\*D (%) – dominance, W – ecological significance

Accidental species	-
Accessory species	<i>A. similata</i> , <i>B. properans</i> , <i>A. viridicupreum</i> , <i>B. quadrimaculatum</i> , <i>B. elegans</i> , <i>C. lunatus</i> , <i>H. tardus</i> , <i>N. brevicollis</i> , <i>P. dejeani</i> , <i>C. germanica</i> , <i>D. germanus</i> , <i>P. niger</i> , <i>T. quadristriatus</i> , <i>C. coriaceus</i>
Constant species	<i>C. fuscipes</i> , <i>H. affinis</i> , <i>S. pumicatus</i>
Euconstant species	<i>H. distinguendus</i> , <i>P. cupreus</i> , <i>H. rufipes</i> , <i>P. melanarius</i> , <i>P. melas</i> , <i>A. carinatus</i> , <i>A. dorsalis</i> , <i>A. signatus</i> , <i>C. fossor</i> , <i>C. cancellatus</i> , <i>C. granulatus</i>

Figure 1. Carabid species frequency in soybean crops in Lukač, Croatia (2016) and the corresponding classification.

In Tovarnik, in eastern Croatia significantly lower numbers of carabid beetles were recorded (Table 3), which supports the results of Virić Gašparić *et al.* (2017). Altogether 253 individuals were collected and classified into 18 species, i. e. about six times less than in Lukač. Eudominant species were *H. rufipes*, with the highest peak of 94 individuals caught in August, and *A. dorsalis* which were more active during May with 31 individuals collected. Two species, *H. distinguendus* and *H. rufipes*, had the highest frequency in Tovarnik, while *Brachinus crepitans* (Linnaeus, 1758), *Calathus fuscipes* subsp. *fuscipes* (Goeze, 1777), *Carabus coriaceus* Linne, 1758, *Laemostenus terricola* (Herbst, 1784), *P. melas*, *Zabrus tenebrioides* (Goeze, 1777) were constant and *A. carinatus*, *A. dorsalis*, *Brachinus elegans* (Chaudoir, 1842), *Calathus micropterus* (Duftschmid, 1812), *Harpalus dimidiatus* (P. Rossi, 1790), *Harpalus tardus* (Panzer, 1797), *P. cupreus*, *Pseudoophonus calceatus* (Duftschmid, 1812), *Pseudoophonus griseus* (Panzer 1797), *P. melanarius* and *Trechus quadristriatus* (Schrank, 1781) were accessory (Figure 2).

*Harpalus rufipes*, a member of the palearctic Carabidae, is a particularly well-studied species with wide geographic distribution and notable services to agroecosystems (Kotze *et al.*, 2011). It is considered as one of the more common species in many cultivated habitats (Loughridge and Luff, 1983). *Harpalus rufipes* overwinters as both larvae and adults; the overwintered adults becoming active towards the beginning of May, with their densities peaking by the end of June (Zhang *et al.*, 1997). Luff (1980) reported activity of adults from April until November, which coincides with our research; this species was present on all four sampling occasions. On both sites (Lukač and Tovarnik), *H. rufipes* had the greatest ecological significance, followed by the *P. cupreus* in Lukač and *A. dorsalis* in Tovarnik. *Harpalus rufipes*, followed by *P. cupreus* and *P. melanarius* were the most abundant species reported from agricultural fields of Eastern European countries by Kromp (1999), which is generally in accordance with our results. Also, previous research from Croatia (Bažok *et al.*, 2007; Igrc Barčić *et al.*, 2008; Kos *et al.*, 2011) support our results; *H. rufipes* and *P. melanarius* were reported as the most abundant species in corn fields.

Table 3. Classification of species dominance and ecological significance of carabid beetles collected in Tovarnik, Croatia 2016.

Species	*D (%)	Classification	W
<i>H. rufipes</i>	57.31	Eudominant	42.98
<i>A. dorsalis</i>	16.21		4.05
<i>C. fuscipes</i>	5.53	Dominant	2.77
<i>H. distinguendus</i>	5.14		3.85
<i>H. griseus</i>	3.95	Subdominant	0.99
<i>P. melas</i>	2.37		1.19
<i>B. crepitans</i>	2.37		1.19
<i>Z. tenebrioides</i>	1.19		0.59
<i>H. tardus</i>	0.79	Recedent	0.20
<i>C. coriaceus</i>	0.79		0.40
<i>P. calceatus</i>	0.79	Recedent	0.10
<i>C. micropterus</i>	0.79		0.20
<i>T. quadristriatus</i>	0.79		0.20
<i>P. cupreus</i>	0.40		0.10
<i>P. melanarius</i>	0.40	Subrecedent	0.10
<i>A. carinatus</i>	0.40		0.10
<i>L. terricola</i>	0.40		0.20
<i>H. dimidiatus</i>	0.40		0.10

\*D (%) – dominance, W – ecological significance

Accidental species	-
Accessory species	<i>A. carinatus</i> , <i>A. dorsalis</i> , <i>C. micropterus</i> , <i>H. dimidiatus</i> , <i>H. tardus</i> , <i>P. cupreus</i> , <i>P. griseus</i> , <i>P. calceatus</i> , <i>P. melanarius</i> , <i>T. quadristriatus</i>
Constant species	<i>B. crepitans</i> , <i>C. fuscipes</i> , <i>C. coriaceus</i> , <i>P. melas</i> , <i>Z. tenebrioides</i> , <i>L. terricola</i>
Euconstant species	<i>H. distinguendus</i> , <i>H. rufipes</i>

Figure 2. Carabid species frequency in soybean crops in Tovarnik, Croatia (2016) and the corresponding classification.

The difference in total collected numbers between the two sites can be explained by differences in weather conditions. Both investigated locations were classified as belonging to the Cfbwx climatic type of the Köppen classification system (Penzar and Penzar, 2000), with temperate/mesothermal climates and where dry winters dominate. Westerly located Lukač is much more humid with lower temperatures than easterly located Tovarnik (Figure 3). In 2016, the two study locations differed in precipitation and soil temperature in the months of carabid sampling. In May, June and August the average sum of precipitation was highest in Lukač, but the average soil temperatures were lower than in Tovarnik, especially in June (DHMZ, 2017). The results of our study on ground beetle populations indicates that abundance increase follows air and soil temperature decrease; ground beetles seem to prefer humid areas and periods with lower air and soil temperatures (Virić Gašparić *et al.*, 2017).

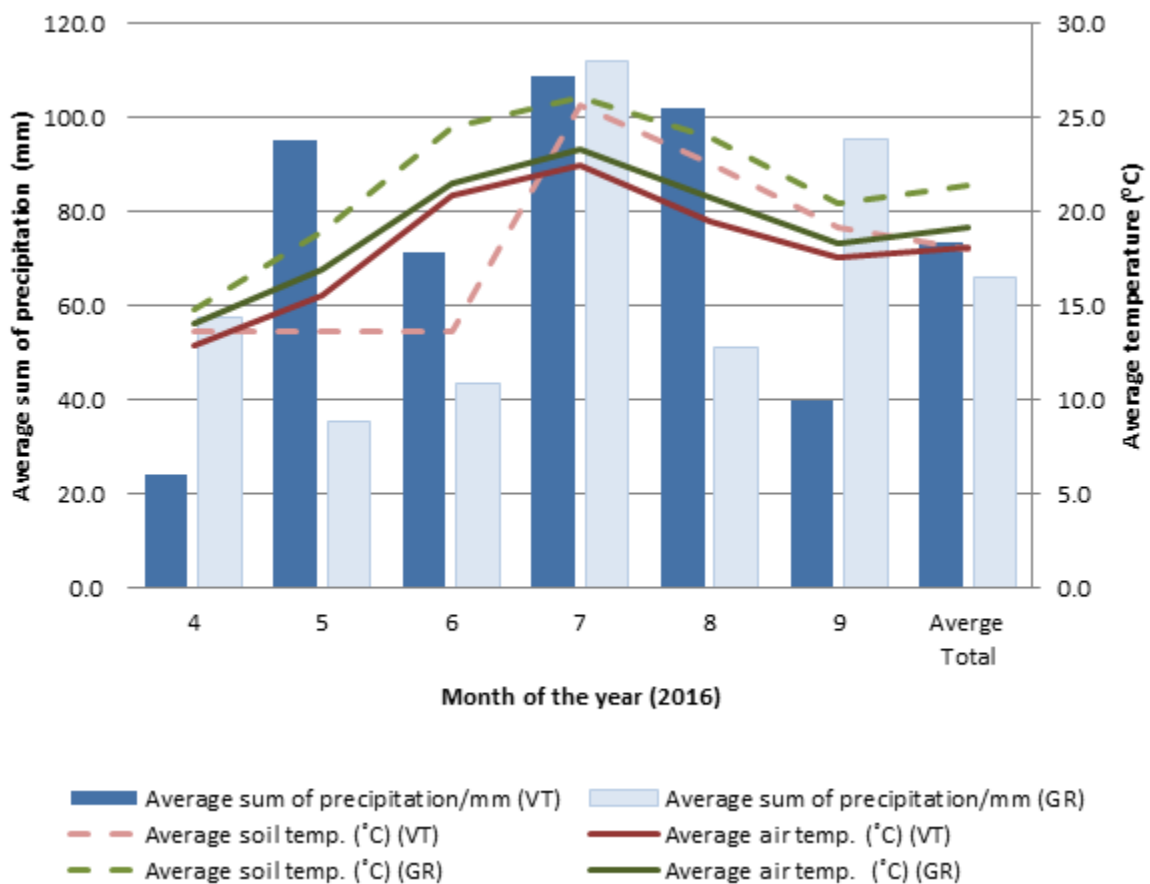


Figure 3. Weather conditions in Lukač and Tovarnik, Croatia 2016.

Ploughing is known to significantly influence soil properties and, along with other factors, affects the abundance of various invertebrates (Vician *et al.*, 2015). Higher ground beetle trapping rates were recorded on fields with reduced tillage or no tillage at all compared with conventionally tilled ones (House & All, 1981; Blumberg & Crossley, 1983; House & Stinner, 1983; House & Parmalee, 1985; Ferguson & McPherson, 1985; Stinner *et al.*, 1988; Tonhasca, 1993), which is in accordance with our results; conservation tillage is common in the Lukač area.

This survey brings the list of 35 determined soybean carabid species of the investigated fields in Croatia which presents a valuable contribution when knowing and preserving beneficial organisms is in question. In Lukač, western Croatia, 1471 individuals were collected and classified into 28 species, while in eastern Croatia (Tovarnik), about six times fewer individuals were collected i. e. 253 individuals classified into 18 species. High temperatures, low amount of precipitation and conventional tillage has again proved to have a negative impact on species abundance in diversity, as we established in previous research. Although soybean is not regularly controlled by insecticides in normal farming practice, knowing the species structure and abundance of carabid beetles as one of the most important predators, could be of great importance in cases of high pest outbreaks as well as decision making regarding insecticide applications.

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**Entomology Session 2:  
Biology, behaviour and control  
of Cabbage Stem Flea Beetle**



## Effect of migration time on population dynamics and damage potential of cabbage stem flea beetle (*Psylliodes chrysocephala* L.)

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**Abstract:** This study aimed to evaluate the influence of different migration periods and beetle densities of *P. chrysocephala* on its damage potential in winter oilseed rape. To simulate different migration periods and beetle densities a field net cage experiment was carried out in which three different beetle densities (20, 40 and 60 beetles/3 m<sup>2</sup>; male:female 1:1) at three different dates (beginning of September, end of September and beginning of October) were released into net cages in 2015/16, 2016/17 and 2017/18 in a winter oilseed rape field at the experimental area of the Julius Kuehn-Institut in Braunschweig, Germany. During the vegetation period different plant parameters and aspects of the biology of *P. chrysocephala* were assessed.

The number of larvae per plant was assessed on three different dates by destructive sampling of ten plants from each cage in early winter (end of November) and spring (January and end of March). Early migration resulted in significantly higher numbers of larvae in autumn but with significant differences between the years (Table 1). In autumn most of the larvae were 1<sup>st</sup> larval instars except at early release of beetles some of the larvae already reached the 2<sup>nd</sup> larval instar. Release at the end of September / beginning of October only generated low larval infestation at the 1<sup>st</sup> sampling. Through the winter until early spring the number of larvae increased significantly in 2015/16 thus it can be assumed that beetles laid eggs until early spring because of mild winter temperatures, whereas the number of larvae in 2016/17 and 2017/18 only slightly increased during winter time. It has to be taken into account that the temperature during winter plays an important role for the population development of *P. chrysocephala*.

High numbers of larvae in autumn significantly influenced the architecture and growth of the plants. The plant height at BBCH 80 was clearly reduced in treatments with 60 beetles released on the early release date (about 15 cm 2016/17 and 30 cm 2017/18) whereas other treatments showed no influence on the plant height. In addition the main stem was damaged which caused a high rate of bushy plants (about 20% in 2016/17 and 50% in 2017/18). An influence of larvae on plant architecture was also found by Nuss (2004). Winter losses of plants only occurred in 2017/18 (about 21% at 60 beetles on the 1<sup>st</sup> release date), but it should be taken into account that the influence of low temperatures might be reduced by the net covering. Thus the winter mortality of plants exposed to frosty temperatures might have been higher under field conditions.

Yield analyses in 2015/16 and 2016/17 only showed differences in 2016/17 in treatments with 60 and 40 beetles and release in early September; a significant reduction (1259 and 431 kg/ha) was observed compared to the control.

Table 1: Mean number of *Psylliodes chrysocephala* larvae / plant in plant samples taken from caged oilseed rape in at a density of 60 beetles/3 m<sup>2</sup> in autumn and spring seasons over three years (small Letters mark significant differences between years for each sampling date and release date; capital letters mark significant differences between release dates for each year and sampling date; Tukey;  $p \leq 0.05$ ).

		Mean number of larvae / plant					
		November sampling			March sampling		
Beetle density	Release date	2015/16	2016/17	2017/18	2015/16	2016/17	2017/18
60/3 m <sup>2</sup>	Early September	3.6 a A	6.1 b A	4.6 a A	9.4 a A	9.1 a A	5.4 b A
	Mid September	2.3 a A	1.5 a B	2.9 a B	9.4 a A	4.4 b B	4.7 b A
	End of September	0.1 a B	0.2 a C	0.5 a C	7.6 a A	1.5 b C	2.5 b B

The data indicate that the temperature during September is very important for the damage potential (= number of larvae per plant before low winter temperature) of *P. chrysocephala*. Early migration and high temperatures during September cause high numbers of larvae during the early growth stages of winter oilseed rape which weakens the plant during wintertime. Late infestation did not show any influence on assessed plant growth parameters and yields. Mathiasen (2015) and Bonnemaïson & Jourdeuil (1954) showed that the beginning of egg laying and the total number of laid eggs depends on the temperature. This study showed that the temperatures in September and the migration date are important for the development of *C. chrysocephala*. In consequence, treatments against late migrating *P. chrysocephala* beetles seem to be unnecessary in German climatic conditions, which is an important fact for damage threshold.

**Key words:** Winter oilseed rape, *Psylliodes chrysocephala*, migration period, beetle density, plant damage, integrated pest control

## Acknowledgements

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## **Population dynamic and sex ratio of cabbage stem flea beetle in Croatia**

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### **Introduction**

After the neonicotinoid restriction by the European Commission (485/2013) which banned the use of imidacloprid, clothianidin and thiamethoxam on flowering crops including oilseed rape, pests that appear in autumn have become an increasing problem, especially the cabbage stem flea beetle (*Psylliodes chrysocephala* L., 1758). Adult forms feed on the leaves and make characteristic 'shot-holing' damage similar to cabbage flea beetles (*Phyllotreta* sp.) but damage is not usually significant. The most significant damage is caused by the larvae that feed inside leaf petioles and stems. They make feeding tubes inside the plant and during winter period can frost causing cracking of plant tissue (Alford *et al.*, 2003; Williams, 2004). To gain an insight into the phenology and occurrence of this pest in Croatia, the aim of this research was monitoring of the pest population and determination of the sex ration of adult forms.

### **Material and methods**

This investigation was conducted during autumn 2017 and spring 2018 on the experimental station of the University of Zagreb Faculty of Agriculture (45°50'59"N 16°11'12"E). Population dynamics of adult forms of cabbage stem flea beetle was monitored using yellow water traps during 2017. Four yellow water traps were placed diagonally in the field on August 29 (before sowing of the oilseed rape on September 9) and were emptied once a week. Monitoring was finished on April 25 at the beginning of flowering (BBCH 63). Adult forms of cabbage stem flea beetle were identified and separated by gender according to differences in first tarsal segment of the front and middle pairs of legs; the first tarsal segment of females is smaller and more regular in size compared to the other segments and the first tarsal segment in the male is triangular-shaped and larger than other segments (Bonnemaison & Jourdeuil, 1954 cit. Cook *et al.*, 2006).

### **Results**

The population dynamic of females and males of cabbage stem flea beetle is shown in Figure 1.

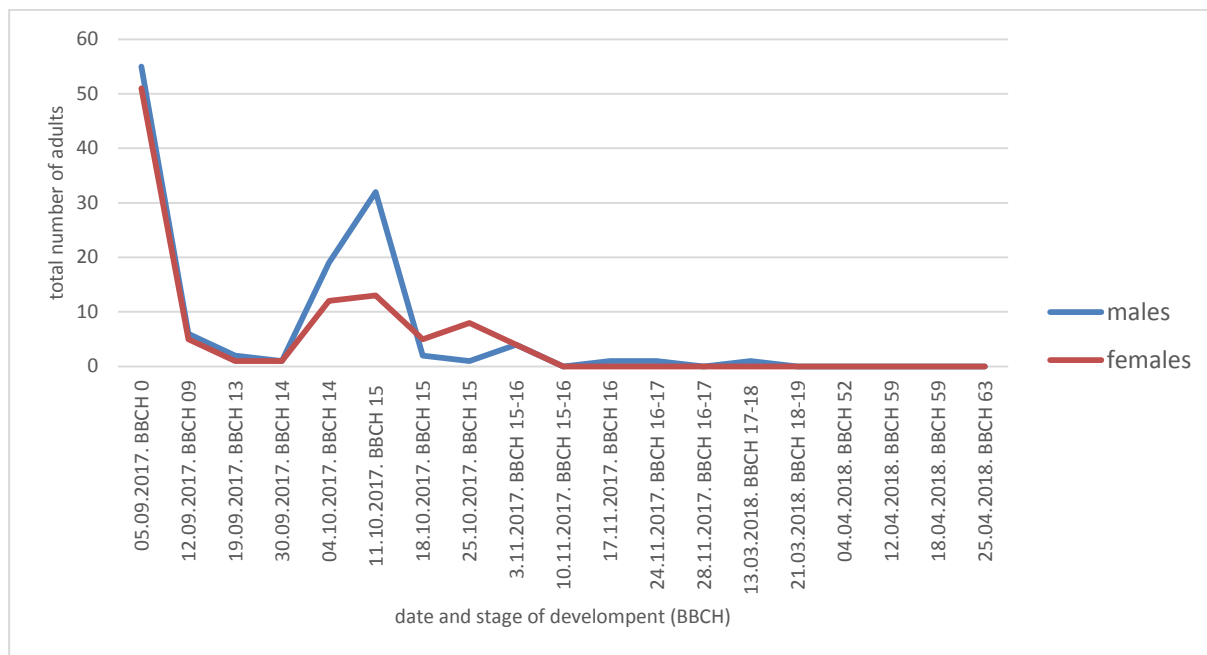


Figure 1. Population dynamic of adult forms of cabbage stem flea beetle during 2017/2018 separated by gender.

During the period of investigation, 225 adult forms of cabbage stem flea beetle were recorded in total. Activity of adults was recorded from September 5 until March 21. The first adults were recorded on September 5, even before sowing of the oilseed rape, which was the peak of the flight at the same time. Second peak flight activity was recorded on October 10 at GS BBCH 15 (Lancashire *et al.*, 1991) (five oilseed rape leaves unfolded). Both genders of cabbage stem flea beetle appeared simultaneously except in the second peak flight activity when higher number of males was recorded.

**Key words:** cabbage stem flea beetle, population dynamic, sex ratio

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## **Investigating non-chemical control options for cabbage stem flea beetle in oilseed rape**

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**Abstract:** Cabbage stem flea beetle (CSFB; *Psylliodes chrysocephala*) remains a serious and intractable problem for winter oilseed rape (WOSR) growers in some parts of the UK. CSFB adults feed on the foliage of emerging crops and can threaten establishment. They lay their eggs at the base of plants and the larvae mine the petioles from mid-October before moving into the stems in spring. Losses due to CSFB in 2016/17 were estimated at 9% of the national crop (> 33,000 ha) (Wynn *et al.*, 2017). In autumn 2016, ADAS (in collaboration with Fera Science Ltd., Cotton Consultancy, Bayer CropScience Ltd. and Syngenta UK Ltd.) began a 40-month AHDB Cereals & Oilseeds-funded project investigating integrated pest management of CSFB in WOSR. The project aims to develop an effective IPM strategy by further understanding the biology of the pest and its relationship with its environment. This paper presents selected results from the first two years of the project. An objective of the project is to assess two non-chemical options for CSFB. The first investigates whether grazing (or defoliating) WOSR during the winter can control CSFB larval populations. The second investigates the use of volunteer OSR (vOSR) as a trap crop for adult CSFB.

### ***Defoliating WOSR overwinter to control CSFB larvae***

CSFB larval feeding can have a significant impact on yield and for several years has been a serious problem for growers. In 2015/16, autumn CSFB larval populations in the UK were higher than in any previous year (Collins, 2017). Large overwinter increases in larval populations have also been recorded in recent years, which may be due to warmer than usual winter temperatures (Collins, 2017), highlighting the potential for climate change to exacerbate issues with this pest. Currently, few chemical control options are available for CSFB larvae, with only pyrethroid foliar insecticides registered for use and targeting these to reach the larvae in the stems and petioles is difficult. An effective control method is urgently required for CSFB larvae in the UK.

This work investigates the potential for grazing or defoliating WOSR during the winter to reduce larval CSFB populations. WOSR is used for grazing livestock in some farming systems (Sprague *et al.*, 2015) and defoliation in the winter prior to stem elongation has been shown to have minimal impact on yield (Spink, 1992; Sprague *et al.*, 2015). This work used mowing to defoliate the crop and mimic grazing. Three mowing timings (December, January and March) and an unmown control (UTC) were investigated. Larval numbers in stems and petioles were assessed by plant dissection prior to each mowing treatment and two weeks after the final mowing treatment. Yield at harvest was also assessed.

Results showed no significant differences in larval numbers prior to mowing. Two weeks after the final treatment, significant reductions in total larval numbers and numbers in the petioles were found in all mowing treatments compared to the UTC. Reductions in larval



number compared to the UTC were greater in later mowing timings than earlier mowing timings (December = 31%, January = 42% and March = 55% reduction in total larvae per plant compared to UTC). The highest yield was recorded in the December mowing treatment (1.8 t/ha) and the lowest in the March mowing treatment (1.4 t/ha) although these differences were not significant. The poor plot yields were due to significant pigeon and CSFB damage over the experimental area. Despite having the lowest larval numbers, plots mown in March had the lowest yield in comparison with all other treatments. This is probably because this mowing occurred after stem elongation. This work suggests that defoliating WOSR during winter but prior to stem elongation may be a cost-effective method of controlling high CSFB larval populations. This work will be repeated in 2018.

### ***Using volunteer OSR as a trap crop***

Trap crop borders consisting of non-OSR Brassicaceae have been shown to reduce CSFB infestation in the WOSR crop (Barari *et al.*, 2005), but drilling and managing these can be expensive. It might be possible to use vOSR as a trap crop for CSFB as the pest has been recorded in vOSR seedlings before the emergence of newly drilled WOSR crops. The technique is dependent on a biological quirk of CSFB adults whose wing muscles are thought to atrophy once they have arrived in a crop and mated (Bonnemaïson, 1965). CSFB that migrate into vOSR may therefore have limited ability to move subsequently into freshly sown WOSR in nearby fields. In the UK, vOSR is usually controlled in August, shortly after the WOSR crop is harvested. This work assessed whether delaying control of vOSR can reduce CSFB incidence and damage in establishing WOSR crops in nearby fields.

Trials occurred at two farms. On each farm two pairs of adjacent fields were selected. In each pair, one field was coming out of WOSR (exOSR field) and the other field was going into WOSR (new OSR). Fields were selected that had similar levels of CSFB pressure in the exOSR fields in the previous year. In one of the exOSR fields the vOSR was controlled in August (standard farm practice) and in the other it was controlled in September (delayed control). The new OSR fields were drilled in the second half of August so that the crops emerged prior to the control of vOSR in the delayed control treatment. Yellow water traps (YWTs) were placed in three assessment areas in the new OSR fields; adjacent to the exOSR field, in the middle of field and furthest from exOSR field. YWTs were also placed in the exOSR fields. The contents of the YWTs were emptied weekly and CSFB counted. To determine the impact of delaying vOSR control on pest damage in nearby WOSR, plant populations and percentage leaf area lost were assessed twice during crop establishment in the adjacent new OSR field.

Results showed that at one farm, numbers of adult CSFB caught between September and October in the new OSR field adjacent to the delayed vOSR control treatment were consistently lower than in the standard farm practice treatment (32-89% reduction depending on week) where the vOSR was removed early. Plant populations in the new OSR were also higher (17-36% increase depending on crop stage) and damage lower (23-27% reduction depending on crop stage) in the delayed control treatment than in the standard farm practice treatment. At the second farm, there was no clear trend in the numbers of adult CSFB and crop damage.

Differences in the effect of treatment between the two sites may have been due to differences in the areas of vOSR in which control was delayed. On the first farm, the control was delayed across the whole exOSR field. On the second farm, it was delayed on approximately a third of the exOSR field, with the remaining vOSR controlled in August (standard farm practice). Results suggest that if control is delayed in a large enough area of

vOSR then this may act to attract CSFB, reducing pest pressure in nearby emerging WOSR crops. This may provide a cost-effective method of reducing pest pressure to tolerable levels. This work will be repeated in 2018.

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## **Ecology and distribution of cabbage stem flea beetle and its parasitoids in UK oilseed rape crops: steps towards Integrated Pest Management**

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### **Background**

The cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala*) is one of the major pests of oilseed rape (OSR) in Europe (Williams, 2010). Adults and larvae feed on the plant during autumn and winter threatening crop establishment and affecting plant vigour, respectively. The pest pressure on this crop has historically been controlled using insecticides. However, since the restriction in the use of neonicotinoid seed treatments in 2013 and the development of pyrethroid resistance by CSFB (Højland *et al.*, 2015), there is currently no alternative control option available for growers. Farmers in the UK have faced complete crop losses in recent years (Wynn *et al.*, 2017) which has meant that growing OSR in some areas has become untenable; resulting in a 21% decrease in the area of oilseed rape grown in UK since 2013 (DEFRA, 2013; 2017). Growing OSR in this current scenario requires the development of new management practices; however, little is known about CSFB interactions with current OSR varieties and their responses to changes in climate factors and agronomic practices.

### **Aims & approach**

Our research project seeks to fill current knowledge gaps and make significant progress in our understanding of the effects of the interactions between management practices, OSR variety and weather on the pest population and their parasitoids. For this, the spatio-temporal distribution of CSFB larvae in England between 2003-2017 will be analysed to provide new insights into their population dynamics. Preliminary analysis of these data has shown a significant increase in CSFB larval number during the past 8 years, although the neonicotinoid restriction does not seem to be the only reason for this. The spatial distribution of parasitoids that attack CSFB larvae will be assessed by recording larval parasitisation levels in crops of winter OSR sampled during spring 2018 from 43 sites across England. Understanding the effects of weather, management practices and OSR varieties on CSFB populations and their parasitoids will enable us to understand and better forecast CSFB population changes and their likely spatial distributions. This project ultimately aims to provide farmers with novel control options that include opportunities to enhance parasitoid populations to provide ecological biocontrol components of an IPM strategy.

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## **Agronomic practices to control cabbage stem flea beetle and rape winter stem weevil**

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**Key words:** rape winter stem weevil, cabbage stem flea beetle, resistance, pyrethroids, agronomic levers

In autumn, cabbage stem flea beetle (*Psylliodes chrysocephala*) and rape winter stem weevil (*Ceutorhynchus picitarsis*) are two major pests in winter oilseed rape. Since 2009/2010, their control has become more and more difficult. It was confirmed that some populations of these two pests are resistant to pyrethroids. Their future management cannot, therefore, rely only on insecticides. Agronomic practices for control are necessary.

### ***Sow early***

Winter oilseed rape (WOSR) must have reached the BBCH14 growth stage when cabbage stem flea beetle (CSFB) infects the crop. From this stage rapeseed has passed its slow growth phase and supports the feeding damage caused by CSFB.

When rapeseed is well developed and grows continuously, the larvae have more difficulty to reach the heart of the plants and damage is reduced. We showed that bushy plants (lacking a terminal raceme due to stem mining larval damage) rarely exceed 20% at flowering when the fresh biomass in late autumn exceeds 1.5 kg/m<sup>2</sup> (Figure 1). This continuous growth is correlated to the early date of emergence and good establishment of rapeseed, which favor the accumulation of biomass and leaf development.

Rapeseed must not suffer from nitrogen deficiency to reach a biomass before winter greater than 1.5 kg/m<sup>2</sup>. In soils where nitrogen may be limiting during autumn, it is possible to intercrop rapeseed with a frost sensitive legume crop, especially faba bean, with rapeseed and/or to add N-fertilizer at sowing.

When soil nitrogen availability is low in autumn, fertilizer (NP or NPK type) can be applied in partial (10 kg/ha N) or full rates (30 kg/ha N) to favor faster growth until BBCH 13-14 stage. These inputs are useless when the rapeseed is poorly established; fertilizer does not recover a badly engaged situation.

Intercropping frost-sensitive legume crops with rapeseed helped to reduce damage of CSFB and RWSW when the biomass of legume crops exceeds 200 g/m<sup>2</sup> (Figure 2). This confirms the results of Cadoux *et al.* (2015).

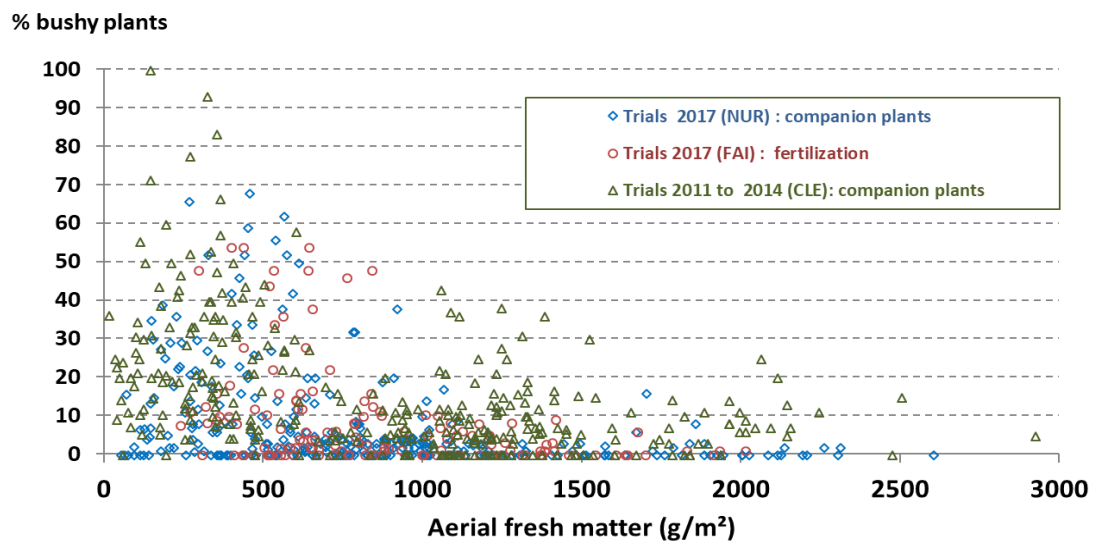


Figure 1. Relationship between aerial fresh matter winter oilseed rape and percentage of bushy (no terminal raceme) plants due to cabbage stem flea beetle and rape winter stem weevil (RWSW).

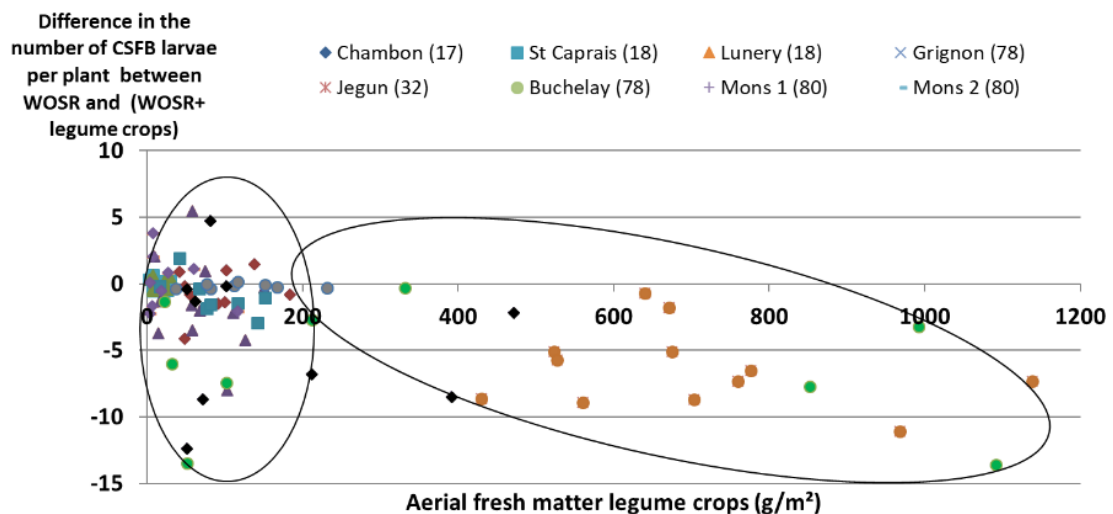


Figure 2. Difference in the number of cabbage stem flea beetle larvae in inter oilseed rape crops with and without companion legume crops according to the biomass of the legume crops.

In the context of insecticide resistance and low efficacy of insecticides, the solution comes from the combination of several agronomic levers and a complementary insecticidal intervention if the growth of the rapeseed is limited and the threshold larvae of cabbage stem flea beetles (2-3 larvae per plant) is exceeded (Figure 3). The choice of the insecticide is reasoned compared to the resistance types present (cartography updated twice a year).

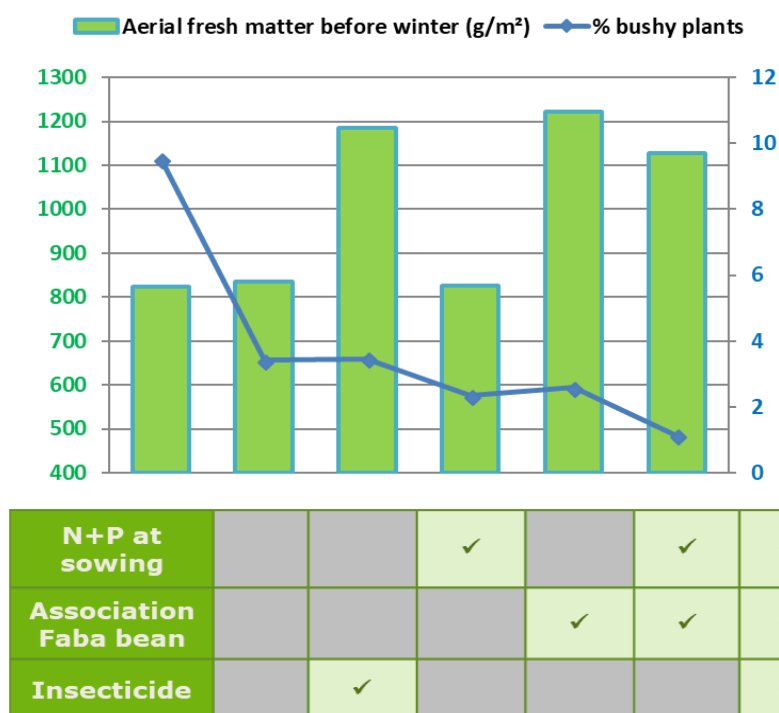


Figure 3. Effect on aerial fresh matter before winter and proportion of bushy plants (as a measure of larval damage) with various combinations of agronomic practices and insecticide treatments (4 trials 2017).

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# **Entomology Session 3: Insecticide resistance**





## **Pyrethroid resistance of insect pests of oilseed rape in Germany**

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**Abstract:** Many insect pests are present in oilseed rape for a long period of time and are therefore exposed to several insecticide applications even though the application is targeted against other pests. Intensive and virtually exclusive use of pyrethroids for pest management in oilseed rape resulted in a selection pressure and finally in resistant insect pest populations. Since 2005, resistance monitoring has been carried out at the Julius Kühn-Institut. More than 1,800 German pollen beetle populations were tested with lambda-cyhalothrin from 2005 to 2017. Also other insect pests of oilseed rape such as cabbage stem flea beetles, different weevil species and the brassica pod midge were tested within the monitoring scheme. The results showed that the percentage of highly resistant pollen beetles increased from 7% in 2005 to 94% in 2017. Etofenprox and tau-fluvalinate still provide some field efficacy but biotest sensitivity is decreasing. Resistance to pyrethroids was also found for the cabbage seed weevil, the cabbage stem flea beetle and the rape winter stem weevil.

**Key words:** resistance monitoring, lambda-cyhalothrin, etofenprox, tau-fluvalinate, insect pests

### **Introduction**

Oilseed rape is known to host a large variety of herbivores. To minimize damage by these, oilseed rape is regularly sprayed with insecticides (Richardson, 2008). In Germany, a mean of about three insecticide applications are used (Freier *et al.*, 2015; Roßberg, 2016). Many of these insect pests are present in the crop for a long period of time and are therefore exposed to several insecticide applications even though the application is targeted against other pests. The main insecticide class used for more than 30 years as foliar sprays in oilseed rape against insect pests are pyrethroids (Nauen, 2005; Heimbach *et al.*, 2006; Müller *et al.*, 2008; Thieme *et al.*, 2010). Products of this mode of action group displaced other insecticide classes caused by low cost, low toxicity to mammals, an acceptable ecotoxicological profile within the EU regulation and because they are easy to mix with fungicides. Farmers often use these insecticides prophylactically without considering the pest threshold values. Because of this frequent and indiscriminate use of pyrethroids and the overlapping appearance of different pest species, some of these insect pests such as pollen beetle (*Brassicogethes aeneus*), cabbage seed weevil (*Ceutorhynchus obstrictus*) and cabbage stem flea beetle (*Psylliodes chrysocephala*) developed resistance to pyrethroids [Heimbach & Müller, 2013; see also Arthropod Pesticide Resistance Database (MSU, 2018)]. In France pyrethroid resistance is also known for the rape winter stem weevil (*C. picitarsis*) (Robert *et al.*, 2015). In Germany two populations of *C. picitarsis* with knockdown resistance (KDR) were found up until now.

In this study the results of the Julius Kühn-Institut pyrethroid resistance monitoring programme of different insect pests of oilseed rape in Germany are presented.

## Material and methods

The development of pyrethroid resistance of insect pests of oilseed rape in Germany has been surveyed since 2005 by a monitoring scheme conducted by the Julius Kühn-Institut (JKI) using the Adult-Vial-Test similar to IRAC Method No. 11 for pollen beetles (IRAC, 2009). In this test the inner walls of glass vials are coated with increasing rates of the pyrethroids lambda-cyhalothrin, tau-fluvalinate and etofenprox. The Adult-Vial-Test was used to test the sensitivity of *B. aeneus*, *P. chrysocephala*, *C. napi*, *C. pallidactylus*, *C. obstrictus* and *Dasineura brassicae*.

Insect pests were collected from all over Germany. Pollen beetles were tested at the JKI or by local experts using vials and a protocol prepared by the JKI. *Psylliodes chrysocephala*, *Ceutorhynchus* spp. and *D. brassicae* were only tested at the JKI. Before the tests, the insects were stored for one night in perforated plastic bags in a climate chamber at 10 °C with untreated oilseed rape inflorescences or leaves as food and water. During the test, 10 insects per vial were exposed at 20 °C and constant lighting. The number of replicates varied according to the number of insects available, commonly four replicates were used. Contrary to the IRAC-Method, the number of affected insects obtained from the 5-hour assessment was used for the evaluation. In most tests an assessment was also carried out after 24 hours. Because pyrethroids show a fast effect, the assessment after 5 hours also seems to be appropriate and has been used frequently. All tests with more than 20% control mortality were excluded from the analysis.

## Results and discussion

More than 1,800 pollen beetle populations were tested with lambda-cyhalothrin from 2005 to 2017. The percentage of highly resistant pollen beetles increased from 7% in 2005 to 94% in 2017. Also the sensitivity to etofenprox decreased rapidly from 2008 to 2017. Pollen beetles also showed a decreasing sensitivity to tau-fluvalinate but in contrast to etofenprox, mortality has remained constant since 2011. The differences in the sensitivity to pyrethroids are also visible in the resistance factors (LD<sub>50</sub>-values of the 10 most sensitive populations/LD<sub>50</sub>-values of the 10 most resistant populations) of lambda-cyhalothrin (nearly 300), etofenprox (53) and tau-fluvalinate (16). For *P. chrysocephala* and *C. obstrictus*, resistance to pyrethroids was recorded in biotests as well as with molecular methods. KDR resulted in resistance factors of about 20 for *P. chrysocephala* but of 70 for *C. obstrictus*. *Ceutorhynchus picitarsis* is spreading into southern Germany. In France pyrethroid resistance, resulting in control failures is well-known. In Germany, 30 *C. picitarsis* populations were tested. The first weevils with KDR were found 2014 in Baden-Wuerttemberg and in Rhineland-Palatinate in 2016. However, up to now, *C. napi*, *C. pallidactylus* and *D. brassicae* still show high sensitivity to pyrethroids.

The number of sensitive pollen beetles decreased continuously over the years and vanished completely since 2010. *Ceutorhynchus obstrictus* and *P. chrysocephala* also developed resistance to pyrethroids. This is probably the result of pyrethroids still dominating the insecticide market with insufficient products with alternative modes of action available. Selection pressure on insect pests is active during most of the spring period, either on the first adults invading the crop or on the larvae later in the year. In contrast to *C. obstrictus* which showed similar reactions to different active substances of pyrethroids (Heimbach & Müller, 2013), differences in the susceptibility of pollen beetles are known. Type I pyrethroids such as

etofenprox or tau-fluvalinate are known to have a higher efficacy against resistant pollen beetles in the field than type II pyrethroids such as lambda-cyhalothrin (Smatas *et al.*, 2012). To allow sufficient control of insect pests of oilseed rape and to prevent further development of resistance, resistance strategies have to be implemented. In Germany the Expert Committee on Pesticide Resistance – Working Group Insecticides, Acaricides developed a resistance strategy for the control of insect pests in Germany (ECPR, 2017). The most effective strategy is to avoid any unnecessary insecticide application. The threshold values should be taken into account and for some insect pests, tools such as yellow water traps should be used. If an insecticide application is necessary, products with different modes of action should be alternated, taking also into account the exposure (= resistance selection) to all other insect pests in the crop.

## Acknowledgements

Part of the work was funded by the Federal Ministry of Food and Agriculture and the Union for Promoting Oil and Protein Plants e.V. Special thanks to the plant protection services of the federal states for supporting the project by collecting insect pests and testing pollen beetles.

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## **Insecticide resistance of the most important insect pests in oilseed rape in the Czech Republic – dissemination of information among farmers**

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**Abstract:** Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ), as an independent Czech state institution, decided to build up a web tool for Czech farmers. Phytosanitary Portal (PP) was launched in 2014 with the aim of making information about Integrated Pest Management (IPM) more understandable for farmers. Important part of PP is Maps of occurrence displaying results of large-scale monitoring of harmful organisms including outcomes of prognostic models. Besides the Maps of occurrence, there are Maps of resistant populations displaying outcomes of research on resistant insect, fungi and weed populations. There can be found results on *Brassicoglyphus aeneus*, *Ceuthorrhynchus obstrictus* (syn. *assimilis*), *Phyllotreta* sp., *Psylliodes cyrysocephala* (= *chrysocephalus*) with tested active ingredients including chlorpyrifos, cypermethrin, indoxacarb, lambda-cyhalothrin, tau-fluvalinat and thiacloprid. Thanks to its expanding content, PP should serve not only for farmers and researchers but also for gardeners and students. We hope that we managed to create an interesting application which can also attract more research professionals. Our current aim is to transfer this application into mobile phones so that the information can be used directly in the field.

**Key words:** decision support systems (DSS), integrated pest management, maps, distribution, resistance, oilseed rape, pests, phytosanitary portal

### **Introduction**

In connection with the obligation arising from the Directive 2009/128 EC on Sustainable Use of Pesticides, the Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ), as an independent state institution, decided to build up a web tool for Czech farmers. Phytosanitary Portal (PP) was launched in 2014 with the aim of making information about Integrated Pest Management (IPM) more understandable for farmers. The theoretical core of PP represents crop-specific guidelines. The practical part is represented by a list of registered plant protection products displayed in colour coding according to their ecotoxicological properties, so-called Semaphore. Another important part is Maps of occurrence, displaying results of large-scale monitoring of harmful organisms including outcomes of prognostic models (SET's for 21 species of harmful organisms, forecast for *Cercospora betae*, *Septoria nodorum* and *Phytophthora infestans*). Maps of occurrence of resistant populations of pests, diseases and weeds represents a newly-incorporated part of PP.

Oilseed rape (OSR) is the second most heavily monitored crop after cereals (approximately 26% of observations). The proportion of OSR sowing area is almost 16% of arable land in the Czech Republic (average proportion OSR is 6.3% in EU) so independent information concerning pest management of OSR pests and diseases is highly demanded by the growers.

## Material and methods

### *Data collection*

Data for application "Maps of occurrence" comes from large-scale field monitoring carried out by the crop protection inspectors of ÚKZÚZ. Data are collected as part of the regular checks of the crops.

There are 1-2 visits per week during the season. Inspectors use visual evaluation as well as a variety of monitoring tools – optical and pheromone traps, light traps, sucking traps, etc.; on average, 60,000 observations are carried out per year. Observations are made both in autumn and spring up until the end of flowering (growth stage BBCH 69).

Data for application "Maps of resistant populations" comes from cooperation with a consortium of five agricultural research institutes (Agritec Plant Research s.r.o., Šumperk; Zemědělský výzkum spol. s.r.o., Troubsko; Mendelova univerzita v Brně, Brno; OSEVA vývoj a výzkum s.r.o., Zubří a Agrotest fyto, s.r.o., Kroměříž). Since 2018, ÚKZÚZ has joined the consortium and started with testing of pollen beetle populations (Figure 2).

Tests follow laboratory methods of Insecticide Resistance Action Committee – IRAC according to the tested insecticide group. In tested active ingredients prevail chlorpyrifos, cypermethrin, indoxacarb, lambda-cyhalothrin, tau-fluvalinat and thiacloprid.

### *Scope of monitoring*

Among the autumn pests dominate *Psylliodes chrysocephala*, *Phyllotreta* spp., aphids (*Brevicoryne brassicae*, *Myzus persicae*); minor pests include *Plutella xylostella*. Spring pests are predominantly *Ceuthorrhynchus pallidactylus*, *C. napi*, *Brassicoglyphus aeneus* and *Dasineura brassicae* with *C. obstrictus* as an occasional pest. During the season the diseases *Phoma lingam* + *L. biglobosa*, *Sclerotinia sclerotiorum* are observed and minor infestations include *Erysiphe cruciferarum*, *Botrytis cinerea* and *Peronospora parasitica*.

For application "Maps of resistant populations", there is list of tested pests, e. g. *B. aeneus*, *C. assimilis*, *Phyllotreta* sp., *P. chrysocephala*. Tests follow laboratory methods of Insecticide Resistance Action Committee – IRAC; specifically the adult vial tests.

## Results and discussion

### *Outcomes of monitoring*

Results of monitoring are displayed within 15 minutes after inserting into the system. Intensity of occurrence is displayed using coloured dots with clearly defined thresholds (Figure 1). If there is available information on the date of treatment, the outcome gets an added value (Figure 3). In addition to the maps of occurrence, there is another tool to improve decision support on first treatments. Short reports are issued, mainly in early spring, to inform growers about the right time for application. These reports are the outcome of cooperation between ÚKZÚZ and research institutes dealing with projects related to OSR pests. These reports specify the date of first application against stem weevils and pollen beetle. This date is

crucial for a given growing season. In connection with climate change, specification of the first treatment is shifting to earlier periods; while the first announcement for the treatment in 2015 was reported in mid-April, in 2017 and 2018, the first announcement was a month earlier (in mid-March).

## Conclusion

For now, PP is becoming an increasingly important tool for decision making regarding a wide range of commodities and their pests and diseases. Thanks to its expanding content, PP should serve not only farmers and researchers but also gardeners and students. We hope that we managed to create an interesting application which can attract more professionals and the public. The current aim is to transfer this application for use with mobile phones so that the information can be used directly in the field.

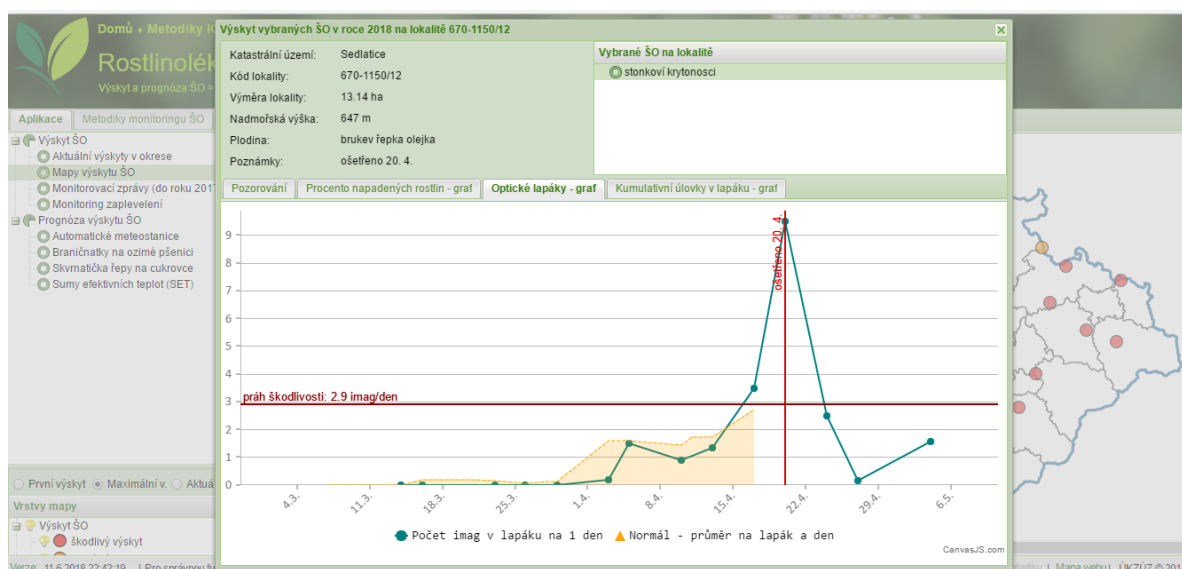


Figure 1. Outcome of PP – catches of stem weevils in yellow traps in selected Czech district (curve shows number of weevils in time, horizontal red line = threshold, vertical red line = date of insecticide application).

Výsledky laboratorního testování v letech: [2011](#) | [2012](#) | [2013](#) | [2014](#) | [2015](#) | [2016](#) | **2017**

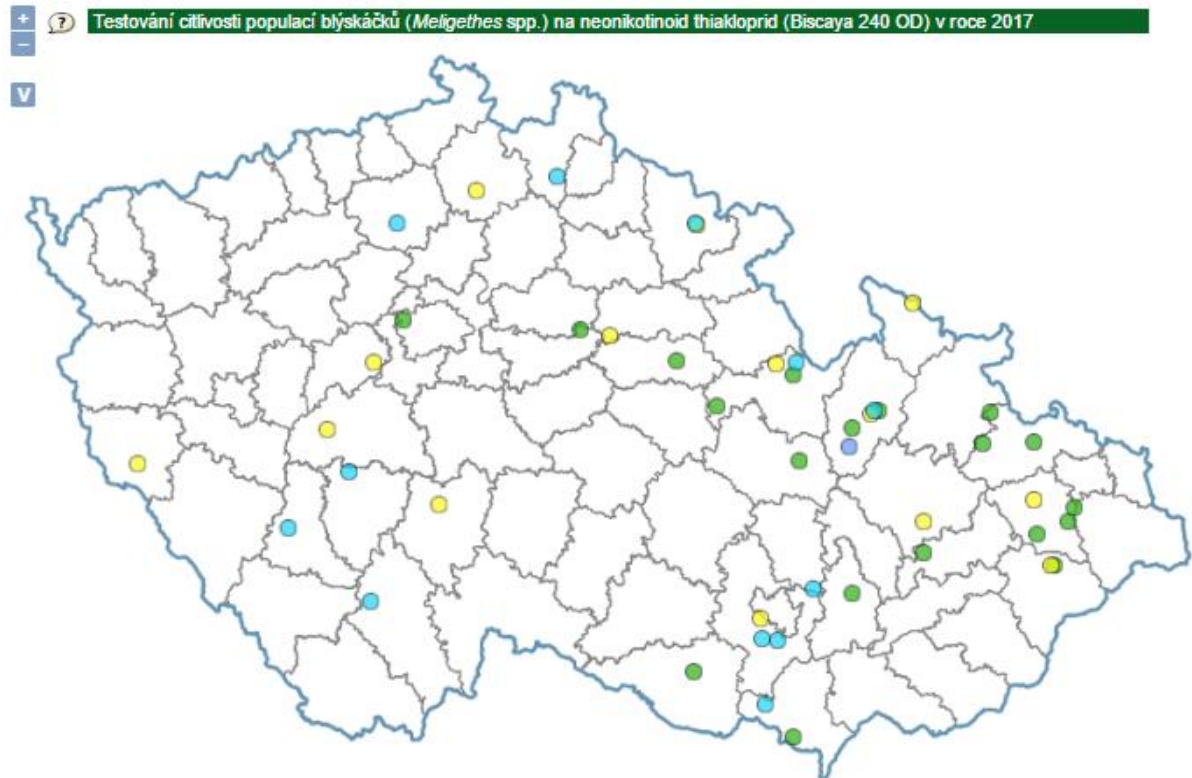


Figure 2. Outcome of PP – Map of resistant populations of pollen beetle to insecticides containing the active ingredient thiacloprid (red = highly resistant, dark blue = resistant, light blue = moderately resistant, yellow = susceptible, green = highly susceptible), tested according to IRAC Susceptibility Test Method 02.

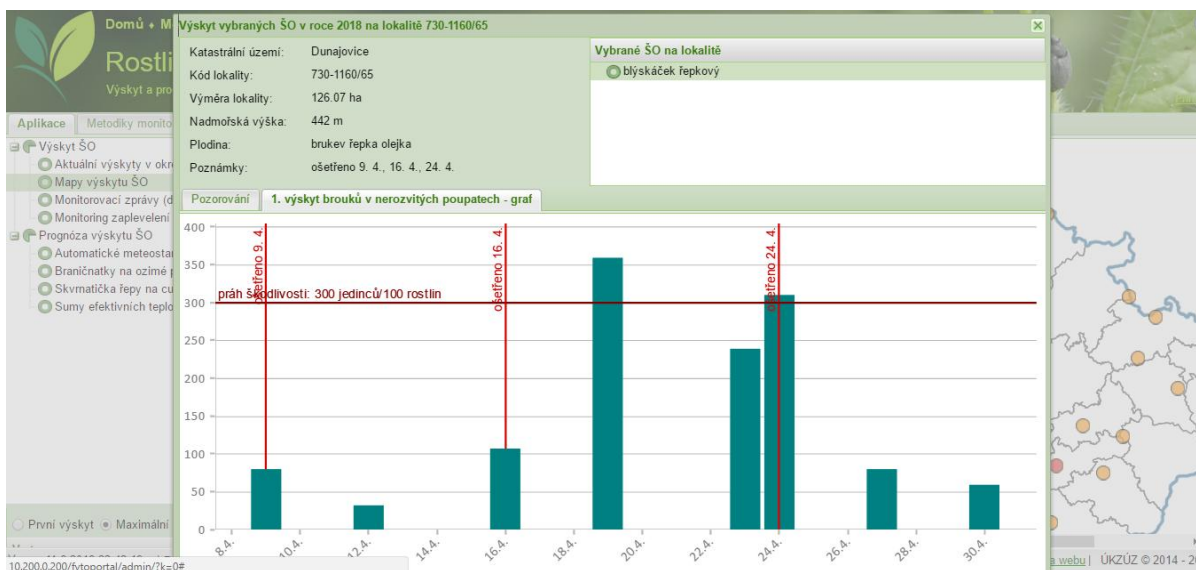


Figure 3. Outcome of PP – number of pollen beetles on plants in selected Czech district (columns show number of beetles in time, horizontal red line = threshold, vertical red line = dates of insecticide application).



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## Description of the situation regarding pollen beetle resistance to insecticides in the Czech Republic and Slovakia

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**Abstract:** Since 2006, the resistance of pollen beetle populations to pyrethroids has been considered as a serious problem in all European countries involved in oilseed rape production and each year the problem is getting worse. In 2017, pollen beetles were collected from 55 locations in Czech Republic and 21 locations in Slovak Republic. The susceptibility of populations was tested to five different insecticides according to IRAC Susceptibility Test Methods. The tested insecticides were lambda-cyhalotrin and tau-fluvalinate from the pyrethroid group, thiacloprid from the neonicotinoid group, chlorpyrifos-ethyl from the organophosphate group and indoxacarb from the oxadiazine group of insecticides. In 2017 most of the Czech and Slovak populations showed resistance (degree 4 according to IRAC) or high resistance (degree 5 according to IRAC) to lambda-cyhalotrin. Relatively high portions of the populations also showed resistance to tau-fluvalinate, the pyrethroid which is, in some cases, perceived as the most suitable pyrethroid for use in situations where pyrethroid resistance is a problem. Some indications of a decrease in contact susceptibility of pollen beetles to thiacloprid have also been recorded in some regions of the Czech Republic and Slovakia. There are populations which have shown significantly different reactions to the active ingredient, which indicates that some of the populations are significantly less susceptible to contact effects of the insecticide. All of the tested populations have shown high susceptibility to indoxacarb – very low doses (mostly 0.20 g a. i./ha) of the insecticide have been fully effective against the Czech and Slovak populations after 24 hours of exposure. All of the tested populations in both countries also showed high susceptibility to chlorpyrifos-ethyl. In most cases doses of about 9 g of chlorpyrifos-ethyl/ha resulted in full mortality of tested populations.

**Key words:** pollen beetle, oilseed rape, pyrethroids, resistance, susceptibility, mortality

### Introduction

The pollen beetle *Brassicogethes aeneus* (Coleoptera: Nitidulidae) is the most commonly-occurring species in the crops of oilseed rape in early spring and occurs in much higher numbers than other *Brassicogethes* species (EPPO, 2007). The adult forms of pollen beetles damage the flower buds of oilseed rape by biting holes in them for feeding, and, to a lesser extent for oviposition and subsequently buds abscise leading to yield loss (blid stalks and fewer pods). The earlier the attack on floral buds occurs the greater the damages because pollen beetle feeding is reduced on buds when flowering starts. Pollen beetle damage can reduce yield of oilseed rape by more than 50% and therefore has been heavily targeted with insecticides over several decades.

Pyrethroids, as a group of insecticides, have been the insecticides of choice because they are effective at the low temperatures which generally occur when risk of pollen beetle is greatest – and they are relatively cheap. After many years of high usage of the same group of insecticides, pollen beetles have developed resistance to pyrethroids. This has become a serious problem in almost every country with oilseed rape production to the extent that future oilseed rape production is affected (Zlof, 2008). Currently, pyrethroid-resistant populations are most dominant in Western and Central Europe and are becoming established in the North and East (Slater *et al.*, 2011). The development of this phenomenon and the progressive spread of pollen beetle populations resistant to pyrethroids through the various countries and regions of Europe has been described and documented in many papers (Derron *et al.*, 2004; Ballanger *et al.*, 2007; Djuberg & Gustafsson, 2007; Tillikainen & Hokkanen 2008; Wegorek 2005; Wegorek *et al.*, 2006; Wegorek *et al.*, 2009; Philippou *et al.*, 2011; Eickermann *et al.*, 2008; Heimbach 2005; Nauen 2005; Heimbach & Müller, 2006; Thieme *et al.*, 2006; Heimbach *et al.*, 2007; Nauen, 2007; Thieme *et al.*, 2008; Heimbach & Müller, 2013). Seidenglanz *et al.* (2015 a, b; 2017) and Stará & Kocourek (2018) documented the spread of resistant populations in Czech Republic (CZ) and also partly in Slovakia (SK) from 2009.

Considering the present-day situation regarding insecticide resistance in pollen beetle the most complicated issue is with description of the susceptibility of pollen beetles to neonicotinoids. There are great differences among the opinions on that. According to the latest published results, significant shifts in pollen beetle susceptibility to thiacloprid (reference active ingredient for the group) were recorded in Europe (Seidenglanz *et al.*, 2018), although in earlier studies respected authors arrived at different conclusions (Zimmer & Nauen, 2011).

In contrast to the situation with pyrethroids European pollen beetle populations seem to be fully susceptible to the organophosphate chlorpyrifos-ethyl, although the active ingredient has also been used for many years in some countries to control insect pests in oilseed rape.

At the same time, European populations of pollen beetles have shown high susceptibility to indoxacarb – a relatively new active ingredient used for control of the insect pest in rape crops.

The objectives of this study are:

- to describe the levels of resistance of pollen beetle populations in the Czech Republic and Slovakia to pyrethroids based of the results recorded in 2017 and to outline the development of this phenomenon during the recent years (between 2009 and 2017),
- to describe the levels of susceptibility of pollen beetles populations in the Czech Republic and Slovakia to the neonicotinoid thiacloprid on the base of the results recorded in 2017 and to outline the changes recorded between 2011 and 2017,
- to describe the results of testing Czech and Slovak populations of pollen beetles to the organophosphate chlorpyrifos-ethyl,
- to report on the baseline susceptibilities of the populations to the oxadiazine indoxacarb.

## Material and methods

Populations of pollen beetles were collected from 55 locations in Czech Republic and 21 locations in Slovak Republic in 2017. Beetles were sampled at different sites by gently tapping flower buds over ae container and by catching them with sweep nets. Adult beetles were transported to the laboratory, put into a ventilated holding cage and left to recover for

24 hours. Pollen beetles were tested to various groups of insecticides according to the IRAC Susceptibility Test Methods. Pyrethroids (lambda-cyhalothrin and tau-fluvalinate) were tested by IRAC Susceptibility Test Method No. 011 – version 3. However one additional concentration (the highest) was used in our tests with lambda-cyhalothrin. The following concentrations were tested: 0 g a. i./ha = untreated control; 0.3 g a. i./ha; 1.5 g a. i./ha; 7.5 g a. i./ha (recommended field rate in Europe); 37.5 g a. i./ha and 112.5 g a. i./ha. The concentrations tested with tau-fluvalinate were: 0 g a. i./ha = untreated control, 1.9 g a. i./ha, 9.6 g a. i./ha, 48 g a. i./ha (recommended field rate in Europe) and 240 g a. i./ha. Neonicotinoids were tested following the IRAC Susceptibility Test Method No. 021. Thiacloprid was used in testing as commercial formulation BISCAYA 240 OD in concentrations: 0 g a. i./ha = untreated control; 2.88 g a. i./ha; 14.4 g a. i./ha; 72 g a. i./ha (recommended field rate in Europe); 144 g a. i./ha. The organophosphate chlorpyrifos-ethyl was tested following IRAC Susceptibility Test Method No. 025. In deviance from the methodology, substantially more concentrations were used in our tests: 0 g a. i./ha = untreated control; 0.09 g a. i./ha; 0.3 g a. i./ha; 0.9 g a. i./ha; 2.9 g a. i./ha; 30.0 g a. i./ha; 96.0 g a. i./ha and 307.2 g a. i./ha (approx. field rate in CZ and SK). IRAC laboratory methods described in detail on <http://www.ircac-online.org/teams/methods/> were fully respected in all other aspects. The oxadiazine group of insecticides (indoxacarb) was tested using IRAC Susceptibility Test Method No. 027. The number of tested concentrations was again increased: 0 g a. i./ha = untreated control; 0.2 g a. i./ha; 0.94 g a. i./ha; 3.19 g a. i./ha; 6.38 g a. i./ha; 9.05 g a. i./ha and 25.5 g a. i./ha (recommended field rate in Europe). After determining the solution volume according to the dimensions of the vials, the amount of active ingredient was calculated and vials were prepared. After the solution was pipetted into the vials, the vials were placed on a roller inside a fume hood and left for one or two hours for acetone to evaporate from the solution. Acetone was used as a control. Ten adult pollen beetles were placed in each vial and vials were stored at 20 °C in a randomised pattern with equal exposure to light. After 24 hours, mortality was tested by placing the beetles on the paper from each vial and by observing their movements. The number of dead beetles, severely affected beetles and the number of live beetles was recorded, and the percentage mortality for each vial and average for the whole population was calculated.

To test for significant differences in the mean percentage mortalities among the compared pollen beetle populations and tested concentrations of the individual active ingredients, analysis of variance (ANOVA) was used with an appropriate post-test (Tukey test). On the basis of mortality recorded in control vials (0 g µg a. i./cm), all mortality figures were corrected according to Abbott's formula (Abbott, 1925). The samples in which the level of mortality in untreated controls exceeded 10% were excluded from assessments. In the most of compared samples the level of mortality was zero. The statistical analysis was performed using Statistica software v.10 (STATSOFT, Inc. 1984-2013). For each population the values of LD<sub>50</sub> and LD<sub>90</sub> were estimated for each of the active ingredients. Probit regression was used (Polo Plus v.2; LeOra Software, Berkeley, CA) for the calculations.

## Results and discussion

### *Resistance of the Czech and Slovak pollen beetles to the pyrethroid lambda-cyhalothrin*

None of the pollen beetle populations showed full susceptibility to the field recommended dose (7.5 g/ha) of lambda-cyhalothrin, except one population (no. 76) from Dravce in eastern Slovakia. In the group of Czech populations, there were only two populations which showed mortality higher than 80% after their exposure to the dose of 7.5 g of lambda-cyhalothrin/ha.

According to the scaling recommended by IRAC (degrees 1-5), there were only resistant (= degree 4: 49.02%) and highly resistant (= degree 5: 50.98%) populations in the Czech Republic. In Slovakia one susceptible population (= degree 2: 4.76%) and one moderately resistant population (= degree 3: 4.76%) were recorded, the rest were again the resistant (47.62%) and highly resistant (42.86%). These results indicate that the situation is very concerning in both countries, although the situation in Slovakia seems to be somewhat less serious than in Czech Republic (Figures 1 a, b). The Figures 2 a and 2 b show the estimated values of LD<sub>50</sub> and LD<sub>90</sub> for lambda-cyhalothrin in Czech and Slovak populations in 2017. In many cases the majority of populations showed higher LD<sub>90</sub> values than the field recommended dose. From Figure 3 a is obvious that the resistant populations began to predominate in CZ in 2010 or in 2011. In comparison to the development of the situation in Germany (described by Heimbach, 2005; Nauen, 2005; Heimbach & Müller, 2006; Thieme *et al.*, 2006; Heimbach *et al.*, 2007; Nauen, 2007; Thieme *et al.*, 2008; Heimbach & Müller, 2013; etc.), the development of the resistance in the Czech Republic was somewhat delayed (about 2-3 years). In Slovakia, the marked increase in the frequencies of resistant and highly resistant populations was further delayed in comparison to the Czech Republic (Figure 3 b). It supports the kind of west-eastern gradient in the phenomenon described by Zimmer & Nauen (2011).

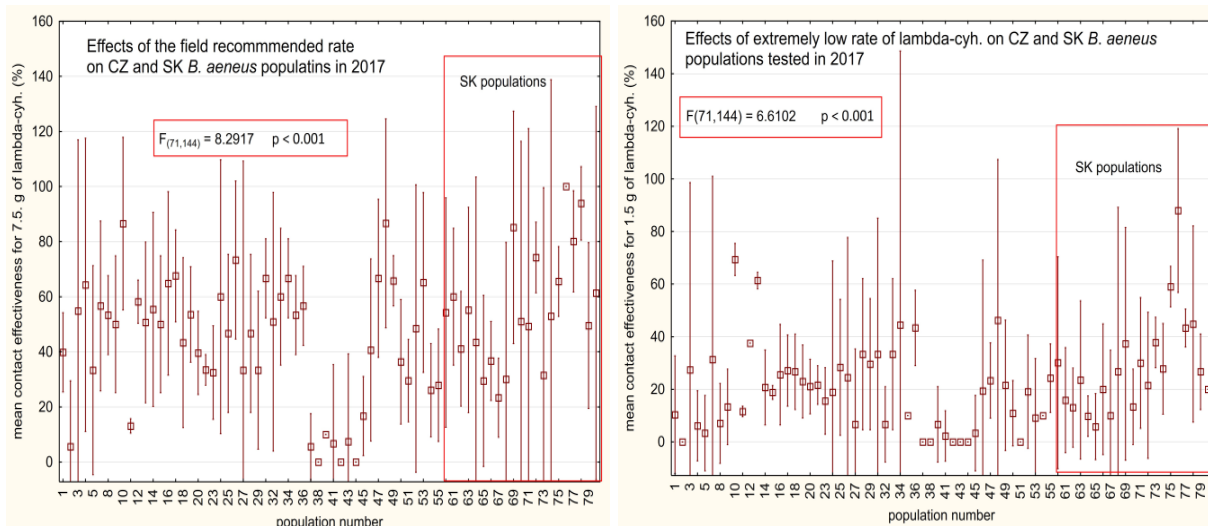


Figure 1. Differences in the levels of mortality corrected using Abbott's formula induced by: (a – left) a dose of 7.5 g lambda-cyhalothrin/ha (= field recommended dose; (b – right) a dose of 1.5 g of lambda-cyhalothrin/ha on Czech and Slovak populations of pollen beetles tested in 2017. **Legend:** brown rectangles = individual populations; vertical line segments = 95% Confidence Limits; populations given within red rectangle frames = Slovak populations.

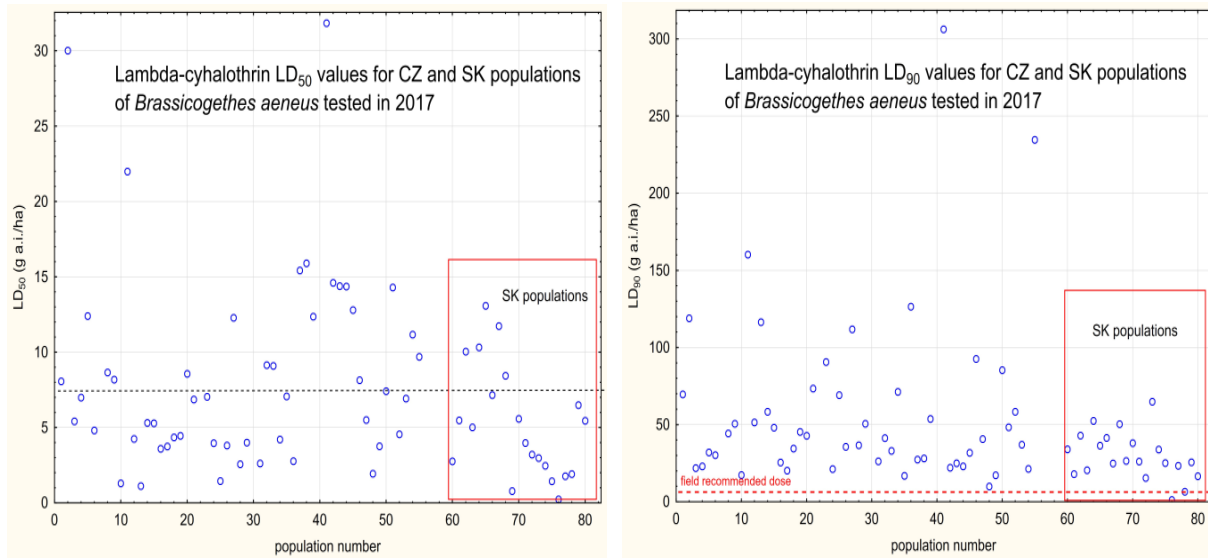


Figure 2. (a – left) Distribution of  $LD_{50}$  and (b – right)  $LD_{90}$  values estimated for the pyrethroid insecticide lambda-cyhalothrin on Czech and Slovak populations of pollen beetles tested in 2017. Legend: blue circles = individual populations; circles within red rectangle frames = Slovak populations; dashed line = field recommended dose (7.5 g a.i./ha).

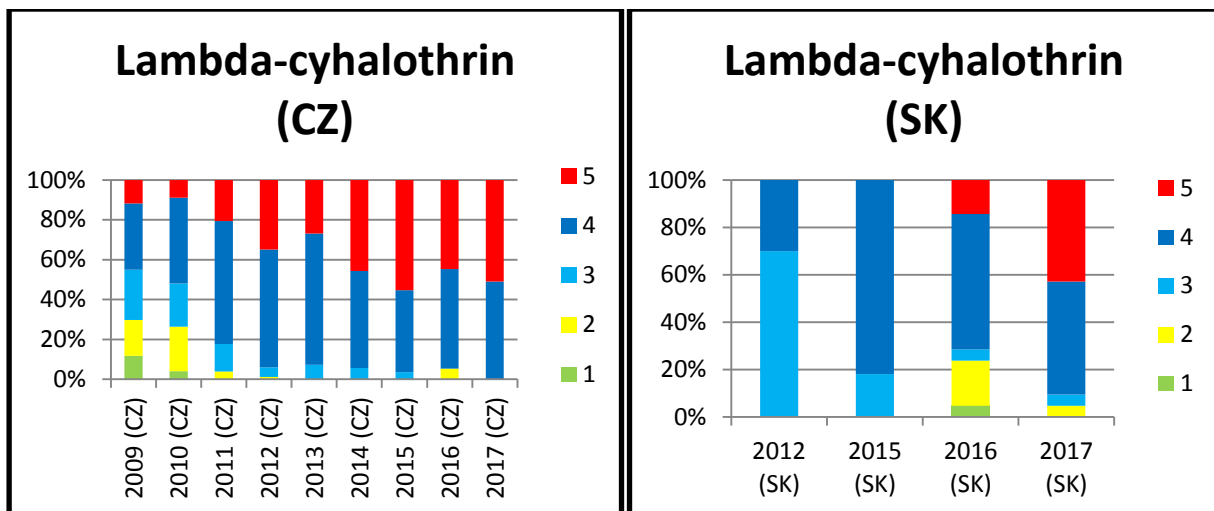


Figure 3. Changes in the proportion of pollen beetle populations showing certain levels of resistance (or susceptibility) to lambda-cyhalothrin in the course of monitoring in (a – left) the Czech Republic (2009-2017) and (b – right) Slovakia (2012, 2015-2017). Legend: degree 1 = highly susceptible population, degree 2 = susceptible population, degree 3 = moderately resistant population, degree 4 = resistant population, degree 5 = highly resistant population; the degrees stated according to IRAC 011, version 3.

### ***Resistance of the Czech and Slovak pollen beetles to the pyrethroid tau-fluvalinate***

The effects of the field recommended dose of tau-fluvalinate (48 g a. i./ha) were markedly higher with tau-fluvalinate than in the case of lambda-cyhalothrin in 2017. On the other hand, the number of populations showing relatively low susceptibility to the effects of the active

ingredient was not negligible in the both countries (Figure 4). The differences among the  $LD_{50}$  and  $LD_{90}$  values were also relatively high (Figures 5 a, b). There were populations recorded with  $LD_{90}$  values exceeding the field recommended dose in both countries (Figure 5 b). But on the base of inter-annual comparisons it is not possible to determine whether or not the situation has been getting worse in the last few years (Figure 6 a, b). Although there are some indications excluding the possibility of cross-resistance between tau-fluvalinate and lambda-cyhalothrin in pollen beetles (Stará & Kocourek, 2017), tau-fluvalinate is not an effective active ingredient for pollen beetle control under field conditions in both countries.

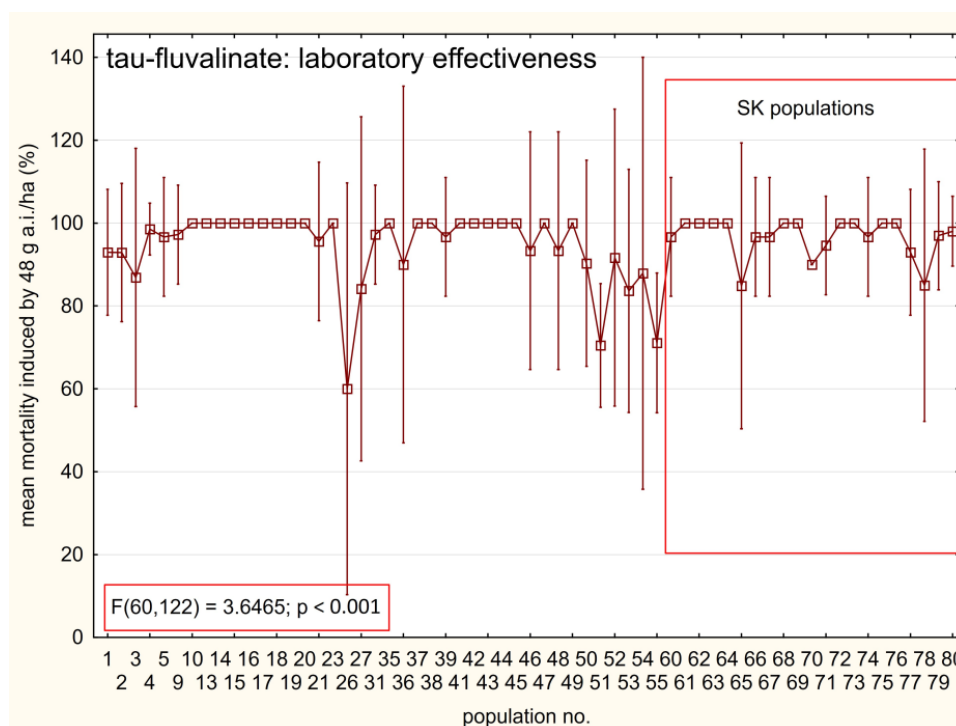


Figure 4. Differences in the levels of mortality corrected using Abbott's formula induced by a dose of 48 g tau-fluvalinate/ha (= field recommended rate) on Czech and Slovak populations of pollen beetles tested in 2017. Legend: brown rectangles = individual populations; vertical line segments = 95% Confidence Limits; populations within red rectangle frames = Slovak populations.

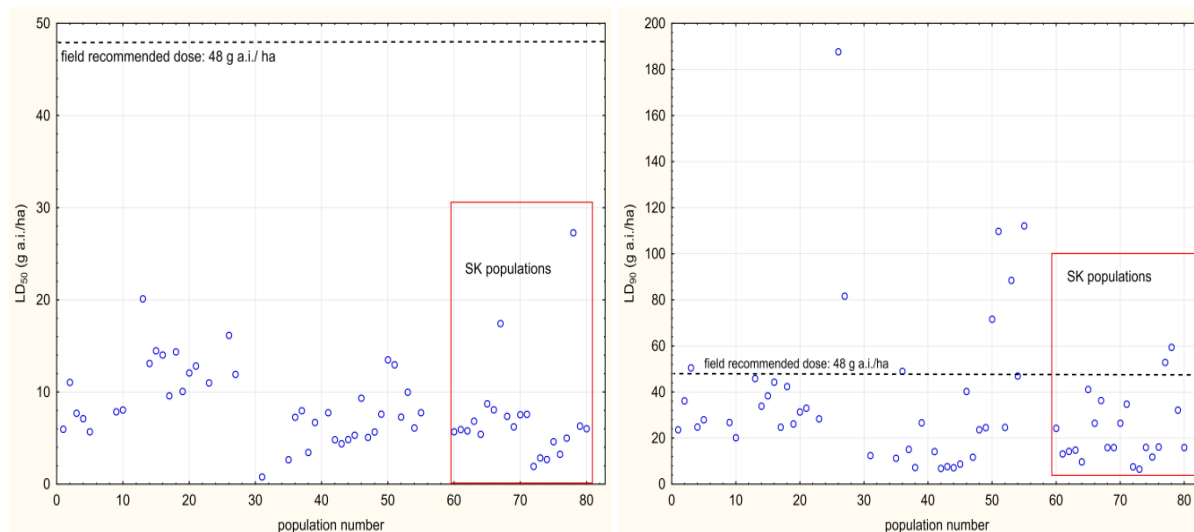


Figure 5. Distribution of LD<sub>50</sub> (a – left) and LD<sub>90</sub> (b – right) values estimated for tau-fluvalinate among individual Czech and Slovak populations of pollen beetles tested in 2017. Legend: blue circles = individual populations; populations given within red rectangle frames = Slovak populations; dashed line = field recommended dose (48 g a. i./ha).

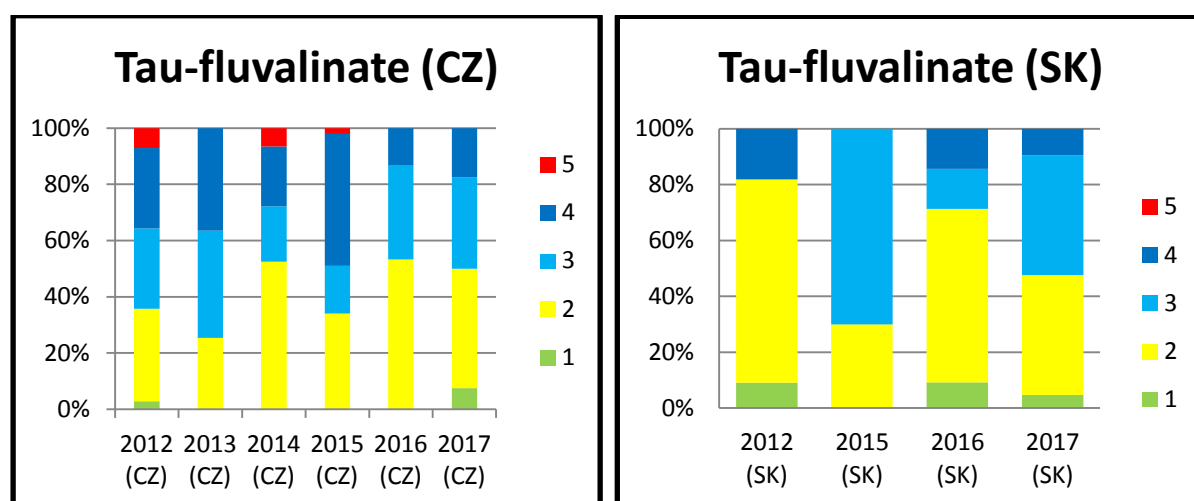


Figure 6. Changes in the proportion of populations showing certain levels of resistance (or susceptibility) to tau-fluvalinate in the course of monitoring in (a – left) the Czech Republic (2012-2017) and (b – right) Slovakia (2012, 2015-2017). Legend: degree 1 = highly susceptible population, degree 2 = susceptible population, degree 3 = moderately resistant population, degree 4 = resistant population, degree 5 = highly resistant population; the degrees stated according to IRAC 011, version 3.

### *Shifts in susceptibility in Czech and Slovak pollen beetle populations to the neonicotinoid thiacloprid*

In both countries, populations of pollen beetles reacted very differently to contact effects of the active ingredient thiacloprid which is assessed through the IRAC Adult Vial Tests. The populations differed in the frequencies of resistant and extremely resistant individuals present.



The difference in the frequencies indicates different reactions of the populations to the active ingredient (Figures 7 a, b) and reveal significant differences among the LD values estimated for the active ingredient in the compared populations (Figures 8 a, b). In contrast to pyrethroids (especially to lambda-cyhalothrin), in the case of thiacloprid there was no difference between the situation in the Czech Republic and Slovakia. The results recorded in 2017, but also in the previous years, indicated that the Czech and Slovak farmers have been threatened with the possibility of pollen beetles resistance to this active ingredient. These results are in strong contrast with earlier, respected studies (e. g. Zimmer & Nauen, 2011). According to our results, thiacloprid is not a suitable alternative for control of pollen beetles in the Czech Republic or Slovakia.

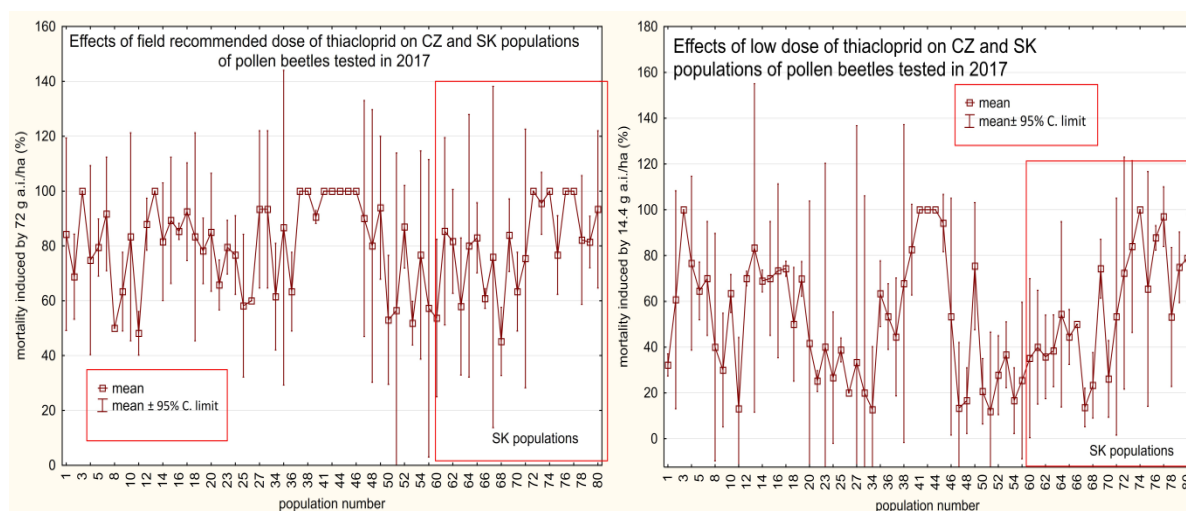


Figure 7. Differences in the levels of mortality corrected using Abbott's formula induced by (a – left) a dose of 72 g of thiacloprid/ha (= field recommended dose: 7 a, on the left) and (b – right) 14.4 g of thiacloprid/ha on Czech and Slovak populations of pollen beetles tested in 2017. Legend: brown rectangles = individual populations; vertical line segments = 95% Confidence Limits; rectangles presented within red rectangle frames = Slovak populations.

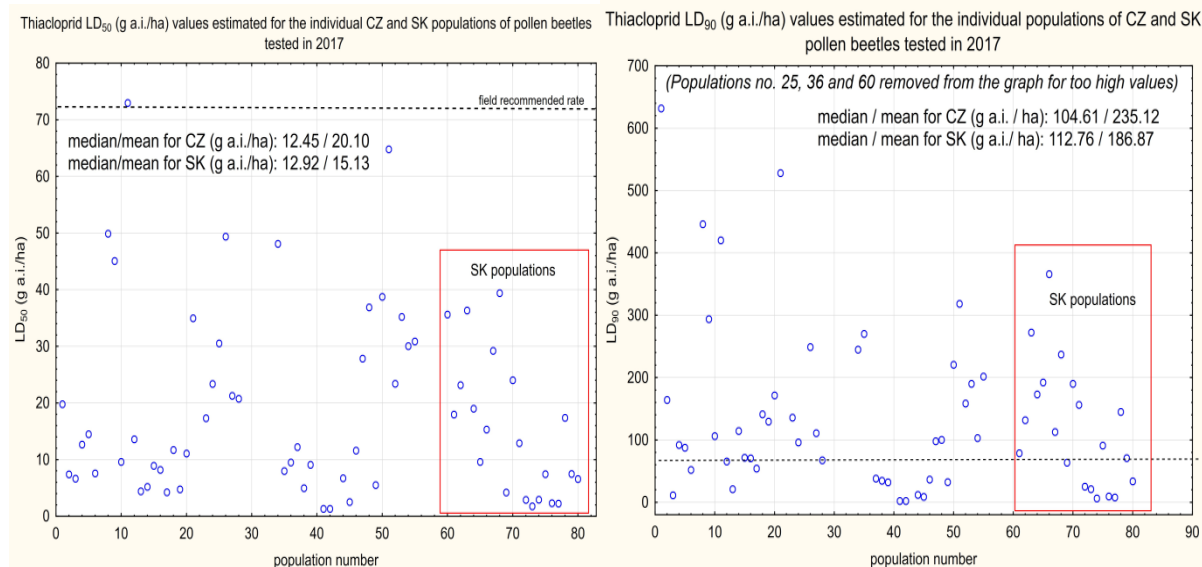


Figure 8. Distribution of LD<sub>50</sub> (a – left) and LD<sub>90</sub> (b – right) values estimated for thiacloprid among Czech and Slovak populations of pollen beetles tested in 2017. Legend: blue circles = individual populations; circles presented within red rectangle frames = Slovak populations; dashed line = field recommended dose (72 g a. i./ha).

### ***Susceptibility of Czech and Slovak populations of pollen beetles to the organophosphate chlorpyrifos ethyl***

Czech and Slovak populations of pollen beetles showed high susceptibility to organophosphate chlorpyrifos ethyl. These results are concurrent with those of Heimbach & Müller (2013) and Wegorek *et al.* (2009), regarding German and Polish pollen beetle populations. All the tested Czech and Slovak populations were fully susceptible to the reference rate (30 g a. i./ha) in 2017. Most of populations were fully controlled with the rates of 0.3 and 0.9 g of chlorpyrifos-ethyl/ha under laboratory conditions. The high susceptibility to chlorpyrifos-ethyl in pollen beetle populations which show resistance to pyrethroids is not surprising. Oxidative enzymes (cytochrome P<sub>450</sub> monooxygenases) play the most important role in detoxification of pyrethroids by insects (Moore *et al.*, 2009; Philippou *et al.*, 2011). A high synergy of pyrethroid insecticides with piperonyl butoxid (an inhibitor of the oxidative enzymes) and low synergy with carbaryl and tributyltin acetate (Wegorek *et al.*, 2007) supports the above statement. However, while with pyrethroids the oxidation results in detoxification of the active ingredients, oxidative desulfuration of chlorpyrifos-ethyl leads to the creation of much more toxic metabolites. A possible predisposition to higher susceptibility to chlorpyrifos-ethyl in pollen beetle populations with lower susceptibility to esteric pyrethroids (higher activity of oxidative enzymes) should be explained by the findings.

Chlorpyrifos-ethyl seems to be a suitable insecticide for use in developing antiresistance strategies but in comparison with pyrethroids, its disadvantage is its high toxicity for bees.

### ***Susceptibility of Czech and Slovak pollen beetles populations to the oxadiazine indoxacarb***

Czech and Slovak populations showed high susceptibility to indoxacarb. In most cases, the LD<sub>95</sub> values for the active ingredient did not exceed the rate of 0.5 g a. i./ha in Czech and Slovak populations in 2017. Populations with LD<sub>95</sub> values slightly exceeding the rate of 1 g a. i./ha (field recommended rate = 25.5 g a. i./ha) were recorded only sporadically. Indoxacarb therefore seems to be a good alternative for controlling pollen beetles in oilseed

rape crops. In comparison with pyrethroids, disadvantages of indoxacarb are its toxicity to bees and very low efficiency on stem weevils, which often occur in crops together with pollen beetles (Seidenglanz *et al.*, 2018).

## Acknowledgements

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## Resistance to pyrethroid insecticides in cabbage stem flea beetle (*Psylliodes chrysocephala*) and rape winter stem weevil (*Ceutorhynchus picitarsis*) populations in France

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**Abstract:** Cabbage stem flea beetle (*Psylliodes chrysocephala*) and rape winter stem weevil (*Ceutorhynchus picitarsis*) management in oilseed rape has become difficult for some years. Available data collected since 2013 show the presence of cabbage stem flea beetle and rape winter stem weevil populations resistant to pyrethroids over the whole oilseed rape growing areas of France. This is probably not a new phenomenon considering the diversity of involved mechanisms.

For cabbage stem flea beetle, a mutation in the sodium channel gene is known to be linked to the knock-down resistance (*kdr*) phenotype was discovered. This mutation is well spread in the French territory. Moreover, another mutation known as super-knock down resistance (*skdr*) was also detected. It is present in the East of France, mainly in the Yonne region. For rape winter stem weevil, the *kdr* mutation was found in the Centre and in the East of France, but not *skdr*. Resistance by detoxification is suspected to be combined with these mutations in the two species.

The first results of this work show that *kdr* mutations would confer a low resistance level to cabbage stem flea beetle. However, there is a good correlation between mortality rate in laboratory and the proportion of individuals with *skdr* mutations.

**Key words:** oilseed rape, *Psylliodes chrysocephala*, *Ceutorhynchus picitarsis*, resistance, pyrethroids

### Introduction

Winter oilseed rape (*Brassica napus*) is visited by numerous insect pests but also pollinators and natural enemies of the crop's pests. In France, the major pests belong to the family of Coleoptera include the cabbage stem flea beetle (*Psylliodes chrysocephala*), the rape winter stem weevil (*Ceutorhynchus picitarsis*), the rape stem weevil (*Ceutorhynchus napi*), pollen beetles (*Brassicogethes* sp.) and the cabbage seed weevil (*Ceutorhynchus obstrictus*).

Oilseed rape pest management mainly relies on insecticide treatments and increasingly on agronomical measures. Today, no effective biocontrol solution is available for these species.

The mean treatment frequency index for insecticide (2.02) represents one third of the treatment frequency index applied on the crop (Agreste, 2014). Since the late 1970s, pyrethroids are the main insecticide group used.

In the previous decades, several oilseed rape pests have been documented as having developed resistance to pyrethroids: peach-potato aphid (*Myzus persicae*) around 1997 (Ballanger, 1999); pollen beetles in 1999 (Ballanger & Detourne, 2011). In recent years, cabbage stem flea beetle and rape winter stem weevil populations have significantly increased. The first species is distributed in regions bordering a sea and in the Centre of France; the second is found almost all over the country. All French regions are concerned with at least one of these two pests.

There are numerous resistance mechanisms to pesticides (R4P Network, 2016). The most common mechanisms involved in insecticides resistance can be classified in three groups (Liu, 2012):

- Increased metabolic resistance: detoxification abilities of resistant insects are increased. The products of three gene families are implicated in the detoxification of insecticides: cytochrome P450 monooxygenases (cytochrome P450s), hydrolases, and glutathione S-transferases (GSTs).
- Target site insensitivity: results from the structural modification or mutation of the target proteins that the insecticides act upon. For pyrethroids, this target is the sodium channel involved in nerve impulse transmission.
- Decreased cuticular penetration or increased sequestration/storage.

In this paper, we will present results on levels of resistance and involved mechanisms in cabbage stem flea beetle and rape winter stem weevil populations in France from 2015 to 2018.

## Material and methods

### *Insect collection*

Since 2013, adults of cabbage stem flea beetle (*Psylliodes chrysocephala*) and rape winter stem weevil (*Ceutorhynchus picipitarsis*) have been collected annually in spring and autumn by a large number of collectors. An amount of 150 to 450 live adults (forming a ‘population’) were collected using yellow traps or sweep nets in a small area (field), stored in aerated container and sending to the laboratory. Between 2013 and 2018, 70 populations of cabbage stem flea beetle and 55 populations of rape winter stem weevil were collected. Since 2015, pests’ larvae were also collected in spring. In a field, between 30 to 50 larvae were extract from plants, stored alive or in ethanol 96% and sending to the laboratory. Sampling was carried out in several regions of France. A few samples came from field experiments testing insecticide efficacy.

### *Fragment of the sodium channel gene sequencing*

Samples were grouped with 10 to 20 insects (mean of 19 larvae or adults) forming each ‘population’. Each insect was lysed for 30 seconds at 6500 rpm with Precellys 24<sup>®</sup> (OZYME), in a 2 ml tube with a 3 mm diameter steel bead. After centrifugation, 100 µl extraction buffer (Tris/KCl/EDTA, Délye *et al.*, 2009) was added. PCR amplification was done using 20 µl; including 5 µl of the lysis reaction diluted at 1/50<sup>th</sup> in ultrapure PCR-grade water, 1 µM of each primer, and 10 µl of AmpliTaq Gold<sup>™</sup> (Life Technologies). Specific primers were used for each species, and the PCR product was sequenced (SANGER method). Amino acid substitutions were detected after chromatograph alignment with reference sequences (sensitive sample) using ChromasPro software (Technelysium Pty Ltd.). The fragment sequenced included the amino acids in positions 906 to 1015 for the cabbage stem flea beetle

and 917 to 1015 for the winter oilseed rape weevil. Between 2015 and 2018, 294 populations of cabbage stem flea beetle and 127 populations of winter oilseed rape weevil were analyzed.

### ***Vial biotests***

$\lambda$ -cyhalothrin, PBO (piperonyl butoxid, P450 monooxygenase inhibitor), DEM (diethyl maleate, glutathione-S-transferase inhibitor) and DEF (S,S,S-tributyl phosphorotrithioate, carboxylesterase inhibitor) solutions were prepared. Accurate dilutions of  $\lambda$ -cyhalothrin in acetone were performed to generate a range of concentrations. Each vial was filled with 200  $\mu$ l of solution ( $\lambda$ -cyhalothrin alone and with inhibitors or acetone alone and with inhibitors as control) and rotated at room temperature until complete evaporation. We had two replicates of each concentration and control and about twenty adult beetles per vial; all were kept at 20 °C with a 12 h:12 h photoperiod. The mortality was scored after 24 hours of incubation.

The concentration of 15 ng/cm<sup>2</sup> of  $\lambda$ -cyhalothrin was tested for each population. This concentration can be considered as a discriminating concentration because it killed over 98% of individuals in the most susceptible populations we studied (M. Siegwart, pers. comm.).

The concentrations of inhibitors used were 50 ng/cm<sup>2</sup> of PBO, 25 ng/cm<sup>2</sup> of DEM and 25 ng/cm<sup>2</sup> of DEF. These three inhibitors were previously tested individually and together, without insecticide, and no induced mortality was observed.

For the largest populations, the number of collected insects was sufficient to test at least four concentrations. In this case, a LC<sub>50</sub> (Lethal Concentration 50, the concentration needed to kill 50% of the population) was calculated using WinDL v2.0 software (CIRAD).

Between 2013 and 2018, 65 populations of cabbage stem flea beetle were analyzed with  $\lambda$ -cyhalothrin (including 28 with inhibitors) and 48 populations of winter oilseed rape weevil were analyzed with  $\lambda$ -cyhalothrin (including 17 with inhibitors).

## **Results and discussion**

### ***Cabbage stem flea beetles***

#### ***Pyrethroids target gene mutation***

Specific mutations on the gene *para* encoding for the sodium channel are known to confer resistance in cabbage stem flea beetles (Zimmer *et al.*, 2014) and other species (Rinkevich *et al.*, 2013). We found these in all analyzed populations (294 populations), except in five populations in eastern France (in the departments of Meurthe et Moselle, Haute Saône, Jura and Rhône).

The most frequent mutation was L1014F, commonly called “*kdr*” for knock-down resistance. It was found in 94% of analyzed populations (Figure 1). This mutation is fixed (100% homozygous individuals) in 5% of populations (in Normandie, Picardie, Ile de France and Poitou-Charente regions).

The M918L mutation commonly called “*skdr*” for super knock-down resistance, was detected in 19% of cabbage stem flea beetles populations. All populations containing this mutation were collected from “Yonne area” (Yonne, Aube, Nièvre, Côte d’Or, Seine et Marne, Loiret and Puy de Dôme – Figure 1). The M918L mutation is always associated with the P909S mutation (not referenced) but never with the L1014F mutation.

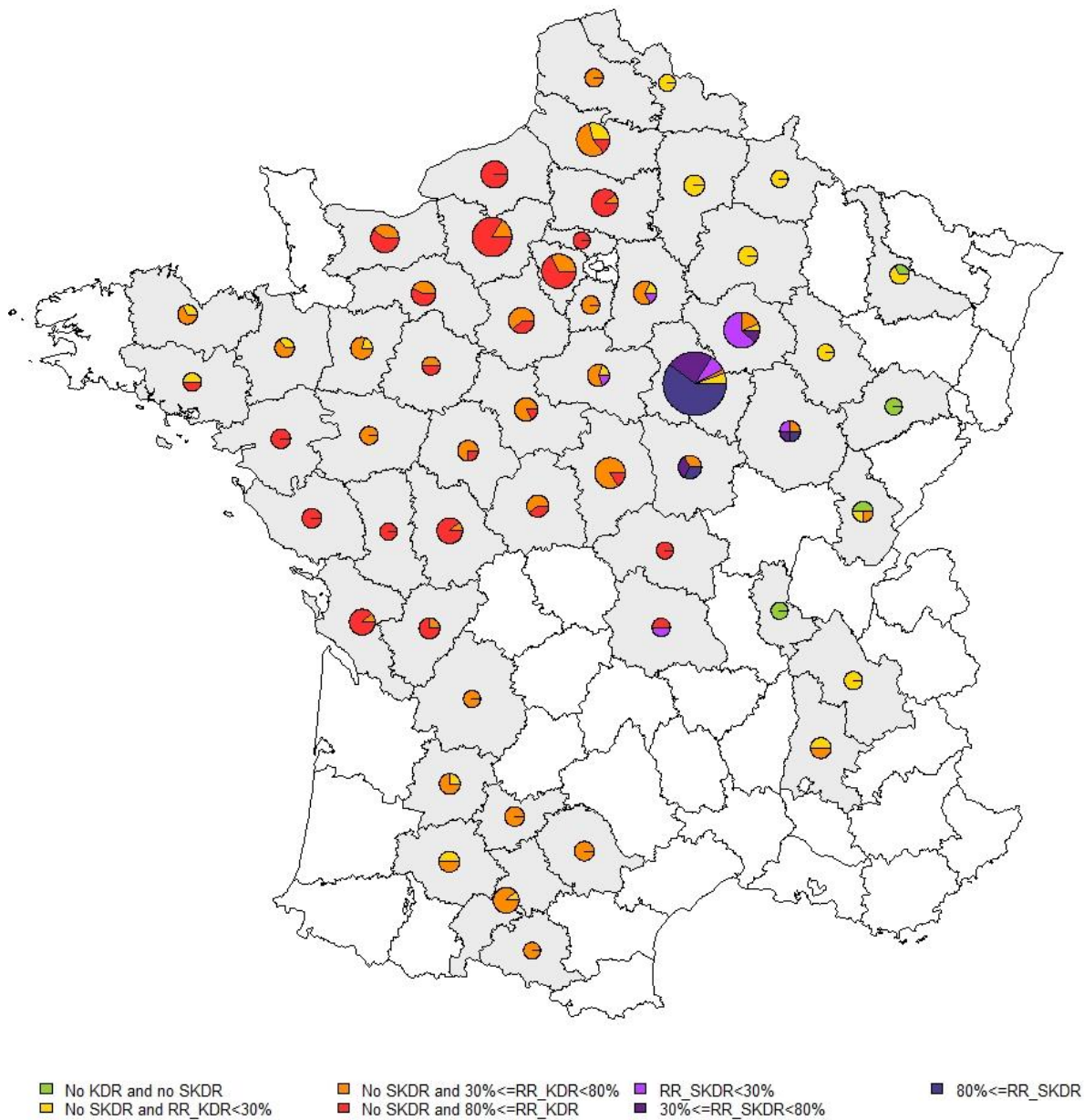


Figure 1. Geographical distribution and frequency of *kdr* (L1014F) and *skdr* (M918L) mutations in cabbage stem flea beetle (*Psylliodes chrysocephala*) populations (France, 2015-2018). The size of the circle is correlated with the number of analyzed populations; between 1-38 populations were analyzed per department, RR = homozygous resistant insects.

Two other mutations listed in the literature for their ability to confer resistance were also detected: T929N (T929I for two populations in Haute Garonne) was described in the coleopteran *Leptinotarsa decemlineata* (Rinkevich *et al.*, 2013) and L925I was detected in a whitefly: *Trialeurodes vaporariorum* (Rinkevich *et al.*, 2013). These mutations were detected alone or associated with another mutation in 34% of tested populations. No geographical relationships were evident (Figure 2).



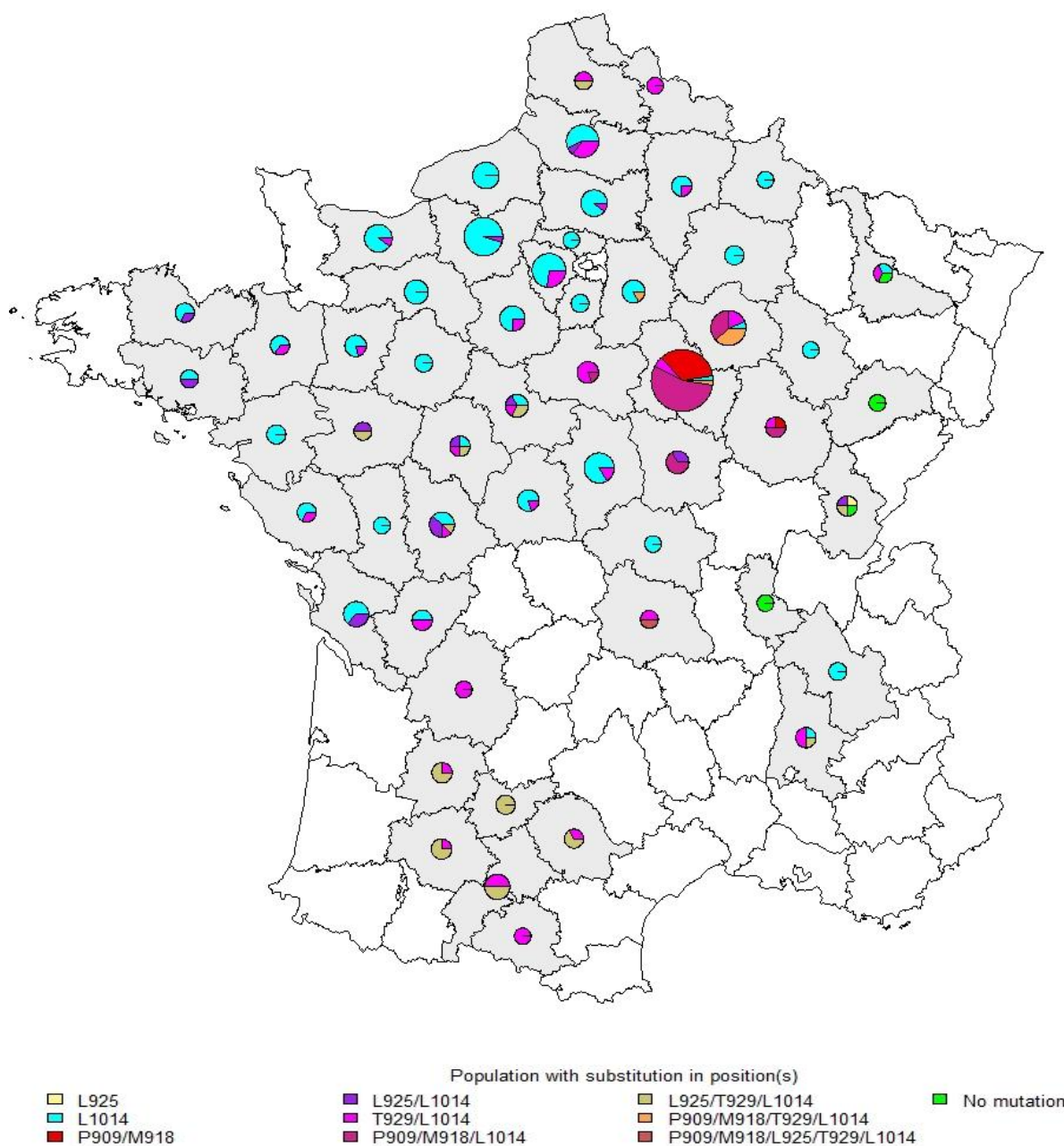


Figure 2. Geographical distribution and frequency of mutations described to confer resistance to pyrethroids in other pests in populations of cabbage stem flea beetle (*Psylliodes chrysocephala*) France, 2015-2018). The size of the circle is correlated with the number of analysed populations; between 1-38 populations analyzed per department.

#### ***Bioassay by tarsal contact on adults (vial tests)***

Vial tests with  $\lambda$ -cyhalothrin were carried out on 65 populations. It was possible to estimate the  $LC_{50}$  for 12 populations. Mortality after 24 hours of exposure at 15 ng/cm<sup>2</sup> (the discriminating concentration) varied between tested populations:

- For populations from the Yonne area, mortality was very low, varying between 0 and 28%. LC<sub>50</sub> was close to 100 ng/cm<sup>2</sup> (Figure 3).
- For populations coming from other areas, mortality varied between 52% to 100% and LC<sub>50</sub> was below 10 ng/cm<sup>2</sup> (Figure 3); i.e. these were more susceptible to pyrethroids than populations from Yonne area.

The LC<sub>50</sub> ratio between the three most susceptible populations (mean  $\approx 1.73$ ) and the three most resistant populations (mean  $\approx 137.55$ ) is 79.5. We therefore conclude that the populations with the lowest mortality rates are resistant to pyrethroids (M. Siegwart, pers. comm.).

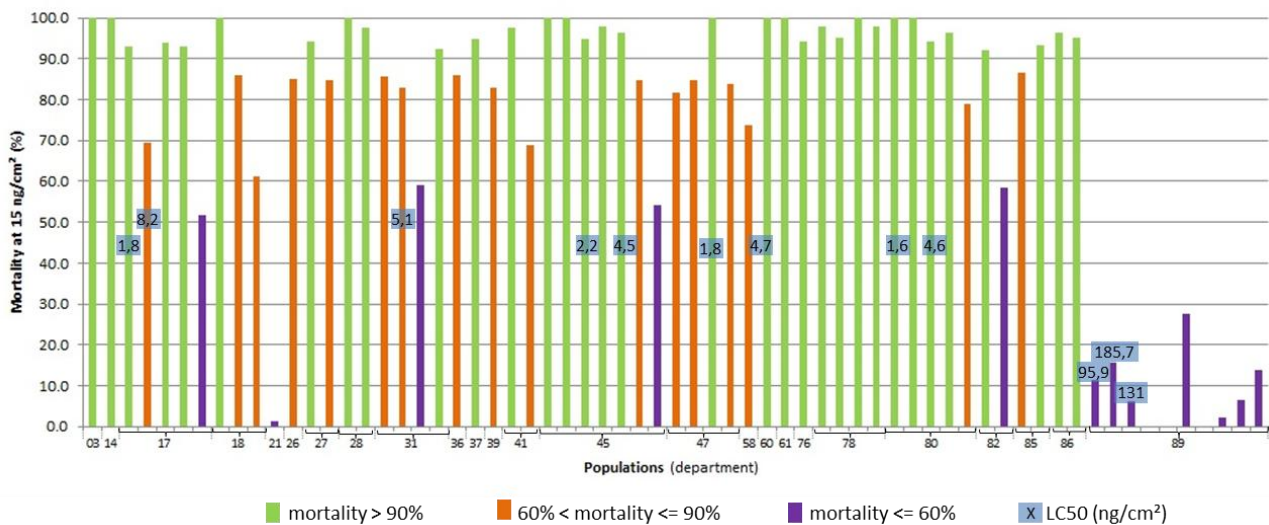


Figure 3. Mortality of cabbage stem flea beetle (*Psylliodes chrysocephala*) populations sorted by department, in vial tests, after 24 hours of exposure at 15 ng/cm<sup>2</sup> of  $\lambda$ -cyhalothrin.

Only three populations among the most susceptible to pyrethroids provided enough insects to carry out vial tests with different insecticide doses and with or without the three inhibitors. For these populations, the difference between the mortality rate with or without inhibitors was around 50% (Figure 4). This difference illustrates the detoxification capacity of susceptible populations. However, if in resistant populations this difference is greater than 50%, we assume that the populations possess metabolic resistance.

In total, 28 populations were tested in vial tests at 15 ng/cm<sup>2</sup> of  $\lambda$ -cyhalothrin with or without the three inhibitors. For most of the populations, metabolic resistance could not be detected because the mortality at 15 ng/cm<sup>2</sup> of  $\lambda$ -cyhalothrin was greater than 50%. For 5 populations, the mortality was very low regardless of whether inhibitors were added or not; other resistance mechanisms are probably involved. For 2 populations located in the Yonne area, this threshold exceeds 50% suggesting the involvement of detoxification mechanisms (Figure 5).

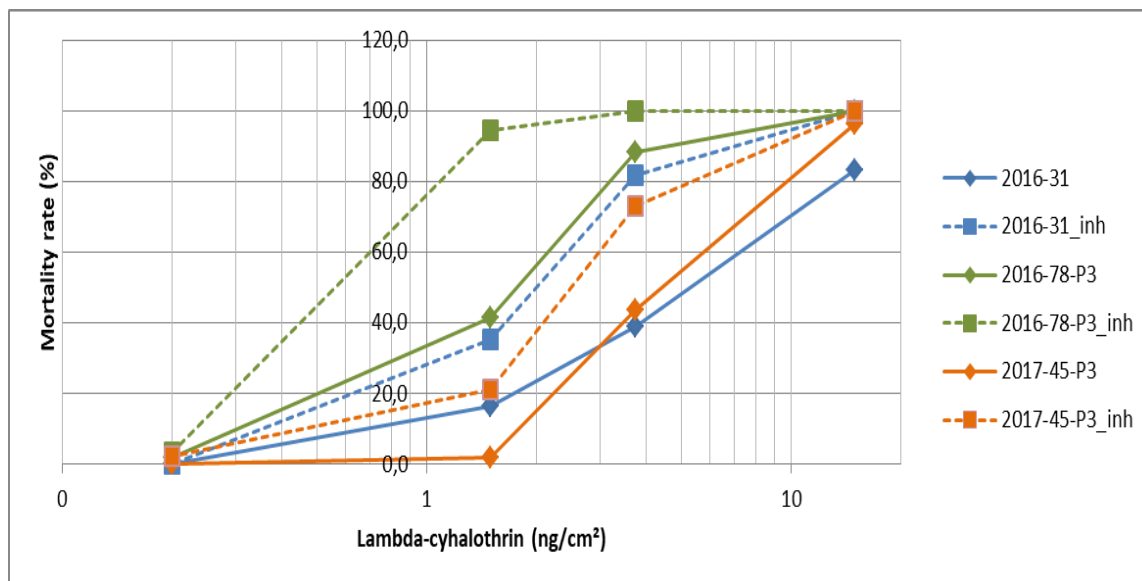


Figure 4. Test dose-mortality after tarsal contact with  $\lambda$ -cyhalothrin with or without inhibitors of susceptible cabbage stem flea beetle (*Psylliodes chrysocephala*) populations.

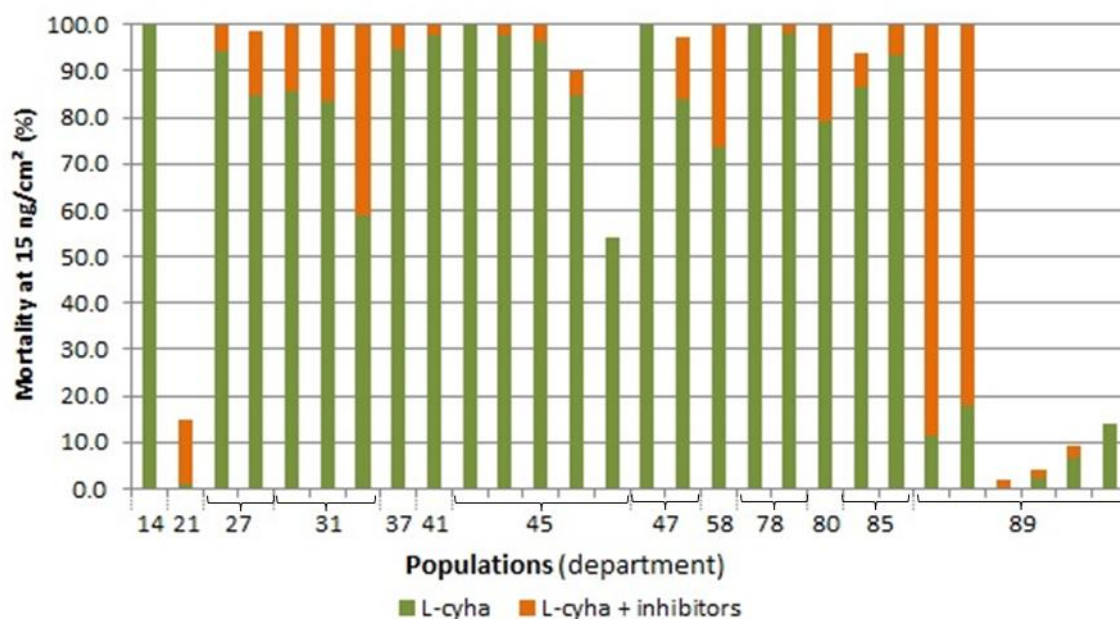


Figure 5. Mortality of cabbage stem flea beetle (*Psylliodes chrysocephala*) populations at the discriminating concentration of  $\lambda$ -cyhalothrin with or without the addition of three enzymatic inhibitors (DEF, DEM and PBO).

#### ***Correlations between bioassays (global resistance level) and molecular analyses (target site mutation)***

In total, 37 populations were analyzed by both bioassays and molecular tests. In these populations, the proportion of homozygous resistant individuals (RR) for *kdr* and *skdr*

mutations varied between 0-100%. Correlation analysis between the proportion of individuals with *kdr* and/or *skdr* mutations and mortality rates in biotests shows:

- a negative correlation between the proportion of individuals with *skdr* mutations and mortality rates ( $R^2 = 0.89$ ; Figure 6).
- In populations without the *skdr* mutation, no correlation between the proportion of individuals with *kdr* mutations and mortality rates ( $R^2 = 0.115$ ; Figure 7).

We unexpectedly noticed that the *kdr* mutation was not the main cause of resistance, unlike the *skdr* mutation which is highly correlated with lower susceptibility. The frequency of the *skdr* mutation was much contrasted: populations carry *skdr* close to 100% or 0% there are very few intermediaries, indicating that it is a very strongly selected marker.

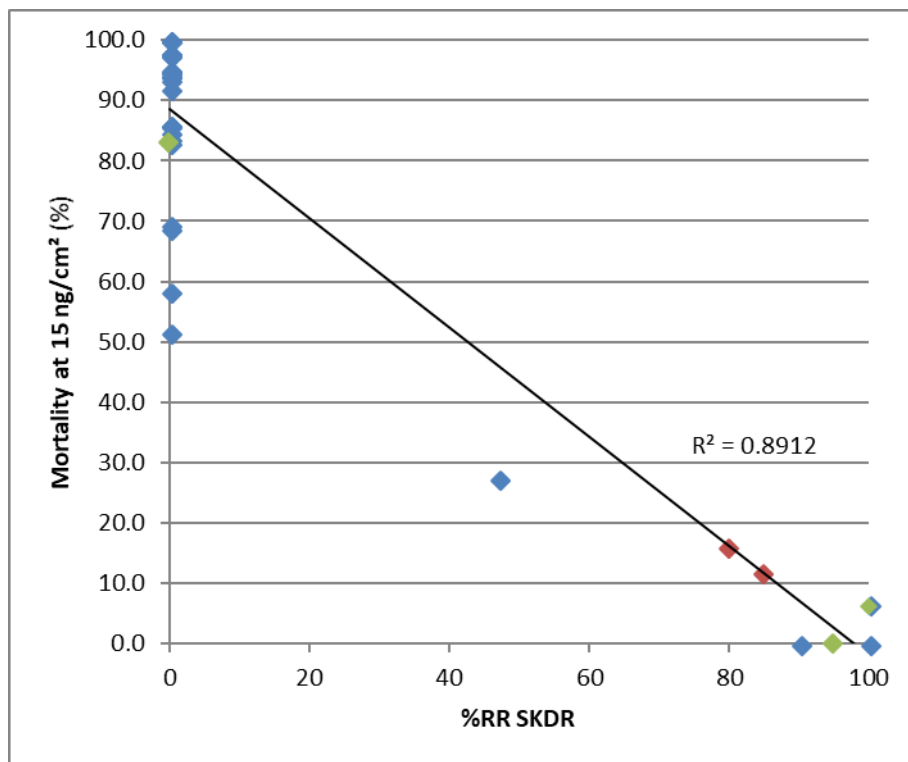


Figure 6. Correlation between *skdr* frequency in populations of cabbage stem flea beetle (*Psylliodes chrysocephala*) and their sensitivity to  $\lambda$ -cyhalothrin at 15 ng/cm<sup>2</sup>. Red: populations in which mortality increase is higher than 50% when inhibitors are added; Green: populations in which mortality increase is lower than 50% when inhibitors are added; Blue: no available information regarding detoxification for these populations.

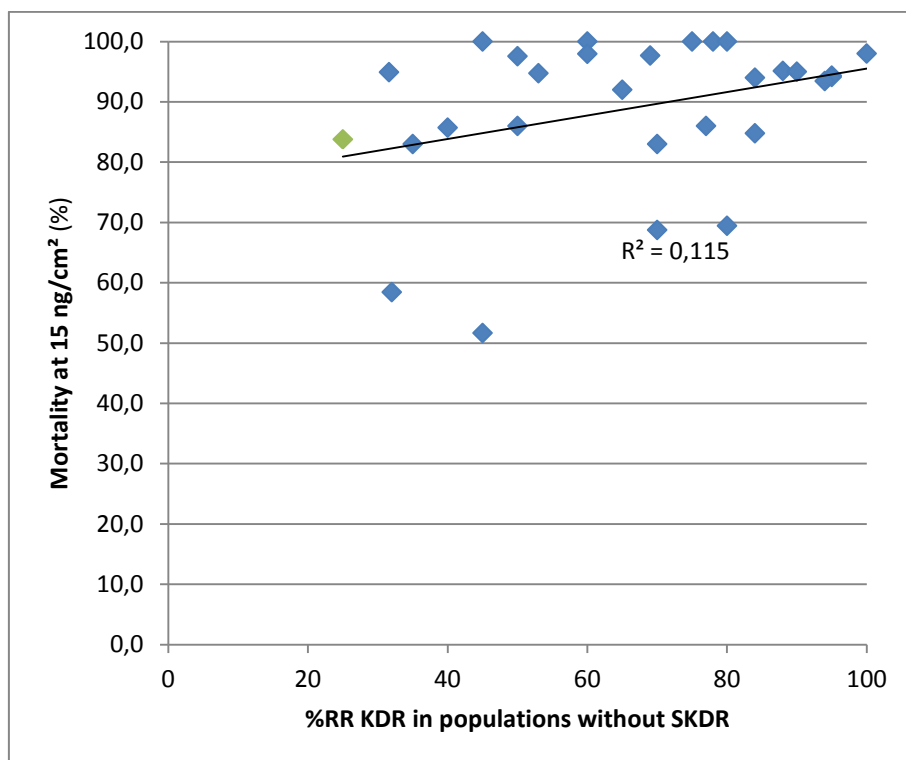


Figure 7. Correlation between *kdr* frequency in populations of cabbage stem flea beetle (*Psylliodes chrysocephala*) without *skdr* and their sensitivity to  $\lambda$ -cyhalothrin at 15 ng/cm<sup>2</sup>. Green: populations in which mortality increase is lower than 50% when inhibitors are added; Blue: no available information concerning detoxification for these populations.

### *Rape winter stem weevil*

#### *Pyrethroids target gene mutation*

Mutations on the gene *para* encoding for the sodium channel and known to confer resistance in other species (Rinkevich *et al.*, 2013) were found in 105 of 127 (83%) analyzed populations. The mutation *kdr* L1014F (L1014H for 2 populations in Aube, one in Côte-d'Or and one in Haute-Marne) was the most frequent; it was found at the heterozygous or homozygous state in 80% of analyzed populations. These mutations were fixed in 21% of populations (Figure 8). Other mutations (L925M and T929N) were found sporadically in a few populations (10 of 127 populations, Figure 9). The mutation M918L (*skdr*) was not found.

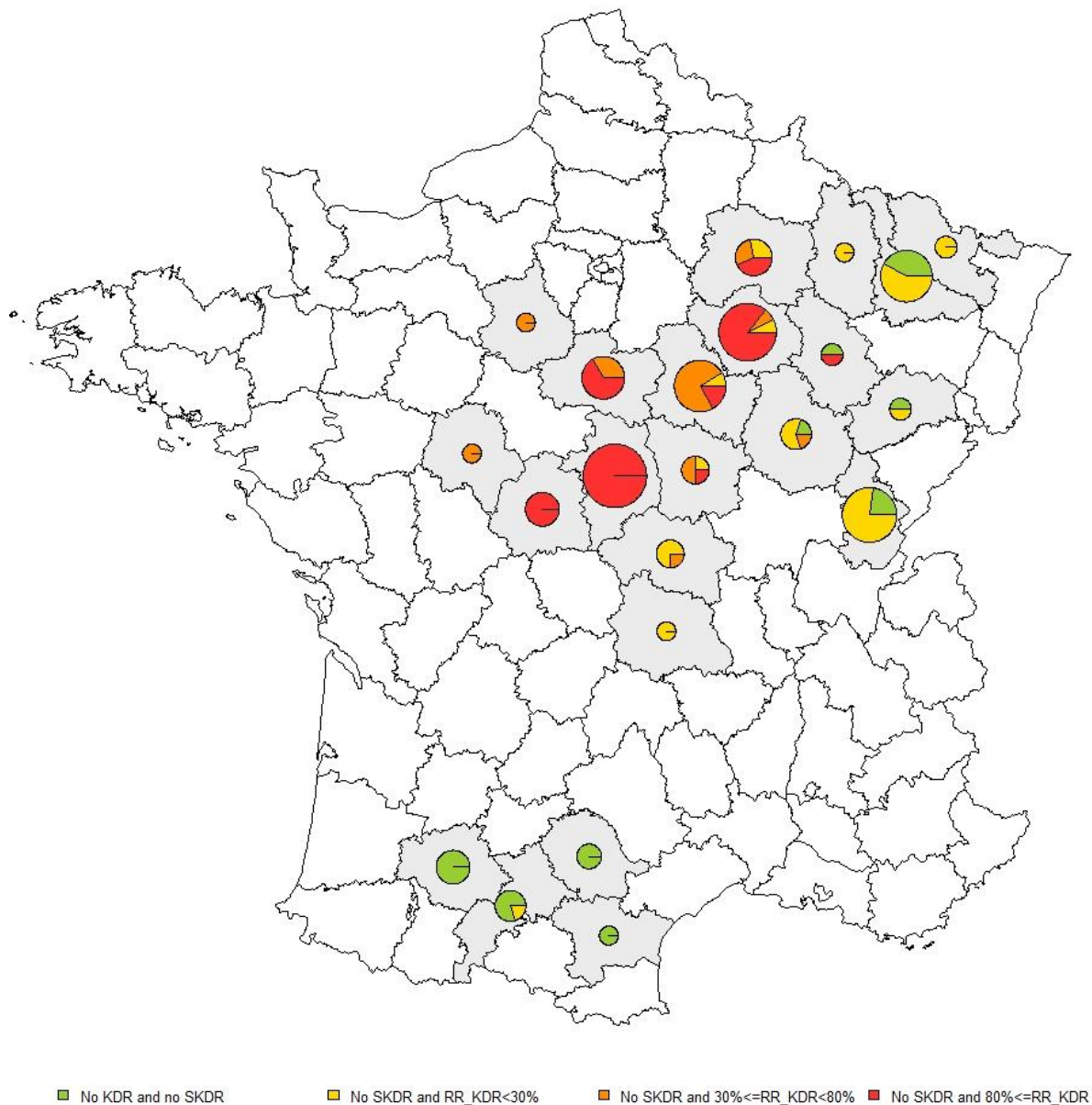


Figure 8. Geographical distribution and frequency of the *kdr* (L1014F) mutation in populations of rape winter stem weevil (*Ceutorhynchus picitarsis*) (France, 2015-2018). The size of the circle is correlated with the number of analysed populations; between 1-16 populations were analyzed per department, RR = homozygous resistant insects.

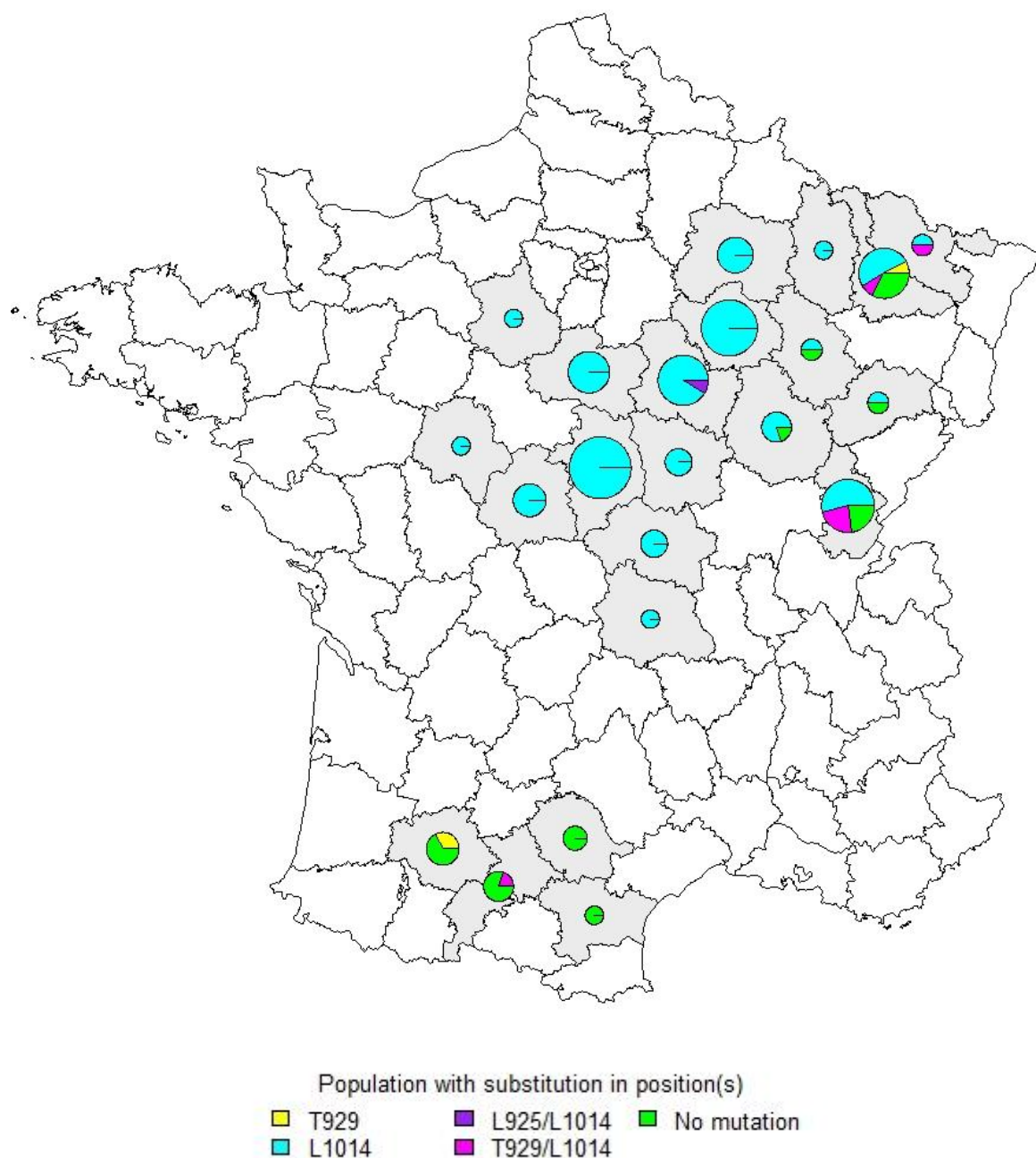


Figure 9. Geographical distribution and frequency in populations of rape winter stem weevil (*Ceutorhynchus picitarsis*) of mutations described to confer resistance to pyrethroids in other pests (France, 2015-2018). The size of the circle is correlated with the number of analyzed populations; between 1-16 populations analyzed per department.

#### ***Bioassay by tarsal contact on adult (vial tests)***

Vial tests with  $\lambda$ -cyhalothrin were carried out on 48 populations. The mortality after 24 hours of exposure to 15 ng/cm<sup>2</sup> of  $\lambda$ -cyhalothrin varied from 12% (the less susceptible populations came from the Cher and the Aube) to 100% (the most susceptible populations came from Puy de Dôme or Gers). It was possible to calculate the LC<sub>50</sub> for 14 populations; this varied from

2.6 ng/cm<sup>2</sup> to 26.1 ng/cm<sup>2</sup> (Figure 10). The LC<sub>50</sub> ratio between the three most susceptible (mean  $\approx$  3.09) and the three most resistant populations (mean  $\approx$  23.28) is 7.5. As it is commonly agreed that the resistance ratio must exceed a factor of 10 to indicate resistance, this means that the less susceptible populations are at least tolerant to pyrethroids.

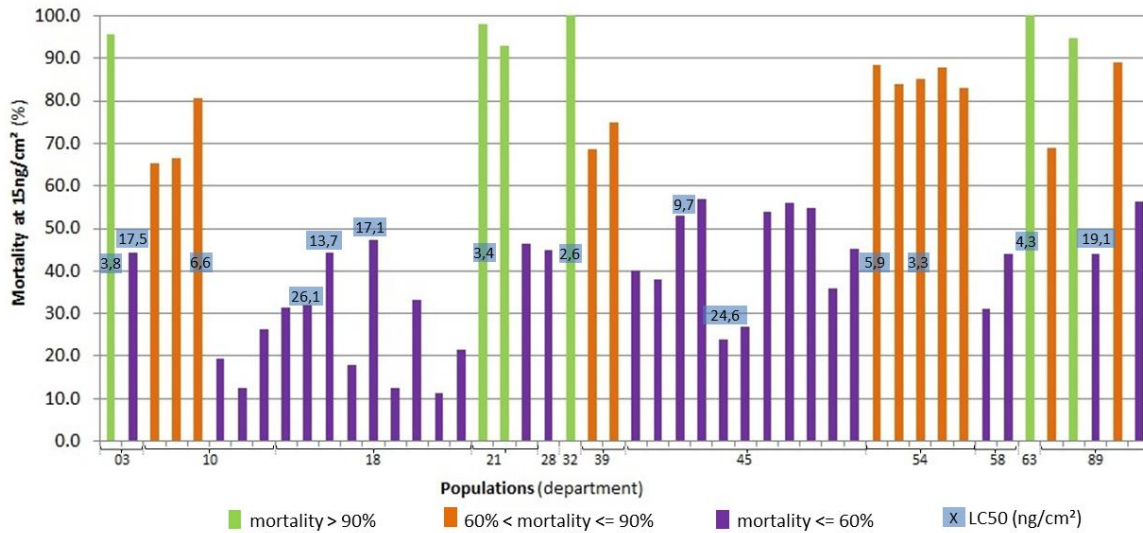


Figure 10. Mortality of populations of rape winter stem weevil (*Ceutorhynchus picitarsis*) sorted by department, in vial tests, after 24 hours of exposure to 15 ng/cm<sup>2</sup> of  $\lambda$ -cyhalothrin.

To determine whether or not metabolic resistance is involved at least in some populations, 17 populations were tested in vial tests with 15 ng/cm<sup>2</sup> of  $\lambda$ -cyhalothrin with or without the three inhibitors (Figure 11). Unlike the case of cabbage stem flea beetle, we were not able to test different doses of insecticide with inhibitors on susceptible populations to estimate the threshold hereafter we could assume that a population would show a metabolic resistance. However, with 15 ng/cm<sup>2</sup> of  $\lambda$ -cyhalothrin and inhibitors, the mortality increase exceeded 60% for 5 populations and was close to 50% for 4 populations. For two populations with intermediary mortality rates, the addition of inhibitors did not increase the mortality. This variability of responses among populations indicates that at least some populations show improvement of their detoxification capacity.

#### ***Correlations between bioassays (global resistance level) and molecular analyses (target site mutation)***

Both bioassays and molecular analyses were carried out on 26 populations. Despite the hypothesis that at least some populations would have metabolic resistance, Figure 12 shows an average correlation between the proportion of individuals with *kdr* mutations and mortality rates in vial tests with 15 ng/cm<sup>2</sup> of  $\lambda$ -cyhalothrin ( $R^2 = 0.71$ ).



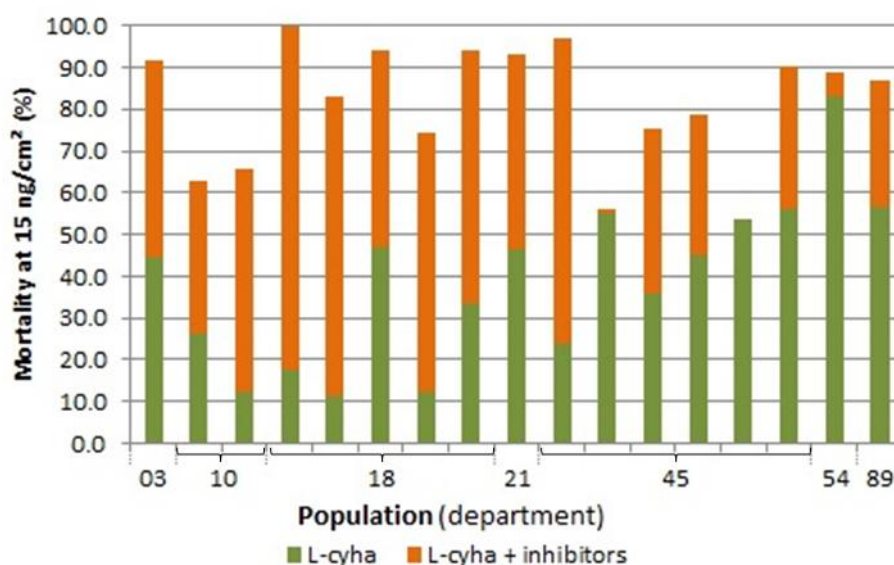


Figure 11. Vial test results to detect detoxification resistance in populations of rape winter stem weevil (*Ceutorhynchus picipitarsis*) in France (sorted by department).

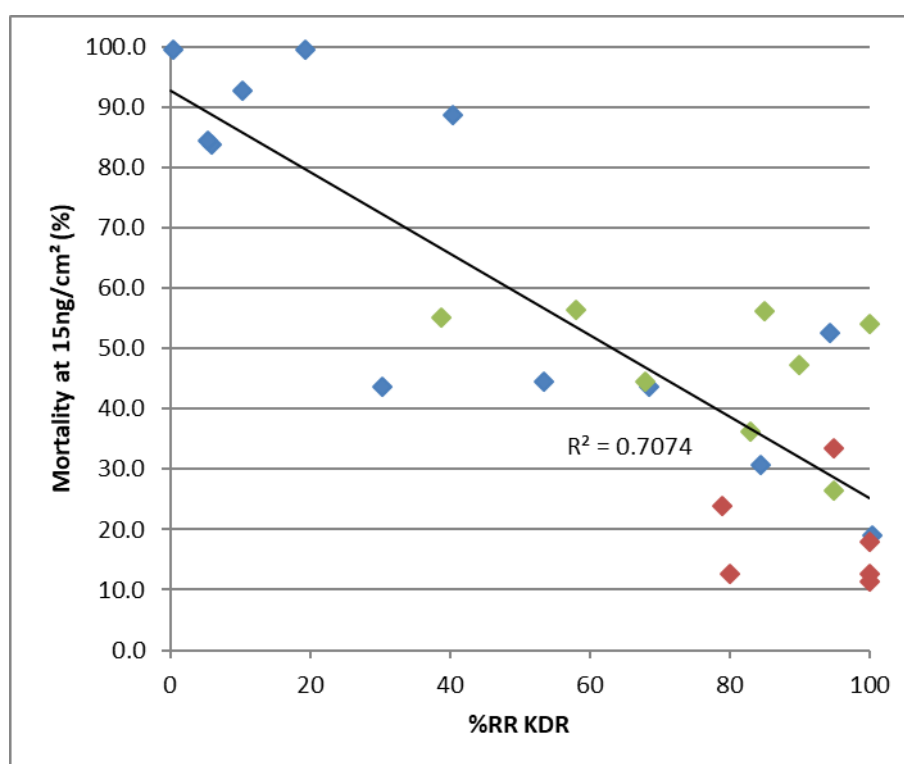


Figure 12. Correlation between *kdr* frequency in populations of rape winter stem weevil (*Ceutorhynchus picipitarsis*) and their sensitivity to  $\lambda$ -cyhalothrin at 15 ng/cm<sup>2</sup>. Red: populations in which mortality increase is higher than 50% when inhibitors are added; Green: populations in which mortality increase is lower than 50% when inhibitors are added; Blue: no available information concerning detoxification for these populations.

## Discussion

The pyrethroid insecticide resistance study on winter oilseed rape pests indicated the presence of resistant populations of cabbage stem flea beetle and rape winter stem weevil to pyrethroids all over the France. The resistance levels vary from one population to another. The emergence of resistant populations is probably not new considering the diversity of involved mechanisms and the magnitude of the phenomenon.

In cabbage stem flea beetle populations, the data show that the phenotype *kdr* is very frequent. The L1014F mutation is the most frequent but other mutations were found. An outbreak of *skdr* phenotype (due to the M918L mutation) was found in the East of France. The area concerned by this problem might spread: new populations are found each year in the surrounding departments. In rape winter stem weevil, the *kdr* phenotype is also very frequent. Few data are available, but they tend to show that the *kdr* phenotype for rape winter stem weevil and the *skdr* phenotype for cabbage stem flea beetle would affect the level of resistance of these two species. However, the tests carried out do not allow to “de-couple” the impact of both resistance mechanisms (target site insensitivity and metabolic resistance) on the global resistance level. We suspect that these two mechanisms can coexist in the same populations. Today, it is difficult to confirm metabolic resistance. For cabbage stem flea beetle we were able to estimate a threshold above which we could assume that a population would show metabolic resistance. More work needs to be done to accurately determine this threshold. However, we were unable to estimate it for rape winter stem weevil. It would be necessary to test several “susceptible” populations with several  $\lambda$ -cyhalothrin doses (plus inhibitors), which was impossible in the current study due to the difficulty in collecting high numbers of adults. It would be even more difficult to class each population according to their level of metabolic resistance for the same reason: it would be necessary to test several insecticide doses on the same population.

Pyrethroid-resistant populations of cabbage stem flea beetle are reported from Germany, United Kingdom and Denmark. In these countries *kdr* phenotype was detected (Heimbach & Brandes, 2016) but the *skdr* phenotype was not found. In the UK, the resistance level is higher and metabolic resistance was also detected (Højland *et al.*, 2015). In Germany, there is a high correlation between the mortality rate after an exposure to  $\lambda$ -cyhalothrin and the proportion of *kdr* phenotype; this is less true in the UK, probably due to the presence of metabolic resistance. As in the UK, our results show a low correlation between the mortality rate after exposure to  $\lambda$ -cyhalothrin and the proportion of *kdr* phenotype, which might suggest the existence of other resistance mechanisms in the same population. According to Højland *et al.* (2015) *kdr* phenotype would confer only a weak level of resistance in comparison with detoxification. However, the diversity of involved mechanisms in cabbage stem flea beetle populations seems to be a French exception.

In our study, we show a good correlation between the mortality after exposure to  $\lambda$ -cyhalothrin and the proportion of *skdr* phenotypes, despite the probable presence of metabolic resistance in some populations. We conclude that in France, the main cause of resistance might be due to the mutation M918L associated with the mutation P909S (not referenced up to now). However, two populations of cabbage stem flea beetles with *skdr* (80% and 85% of RR\_SKDR in these populations) are suspected to have metabolic resistance. Our first hypothesis to explain this result, is that bioassay results (vial test with  $\lambda$ -cyhalothrin and inhibitors) are invalid, even if we were not able to detect where the problem was. Our second hypothesis is that an unknown resistance mechanism associated with *skdr* (for example another mutation not detected) is involved in conferring resistance in most populations of the Yonne area, but not in the two populations in which we suspected metabolic resistance.

Currently, data are insufficient to link precisely the loss of efficiency observed in field experiments and laboratory measures. Since 2014, 15 field experiments testing insecticides efficacy have been conducted by Terres Inovia and showed large variability of average efficiency of a deltamethrin (reference pyrethroid) treatment in November to reduce larvae of cabbage stem flea beetle populations (from 0 to 75% decrease in larval numbers per plant at the end of winter, with a mean of 41%). However, none of the laboratory analyses were conducted on these insect populations because of the difficulty to collect samples. So, it is impossible to link the observed variability in effectiveness in the field to the intrinsic resistance (target site insensitivity or detoxification increase) of the insects, or other factors as treatment application (time, conditions, activity...) or pests' physiological state (vulnerable life stage). The cabbage stem flea beetle populations are difficult to manage in the Yonne area; plants are heavily attacked, insecticide treatments are ineffective and/or are not sufficient to prevent crop damage. These field results as well as farmers and stakeholders' alerts match the results from our laboratory bioassays and suggest that the resistance level must be very high in this area.

Monitoring the appearance and spread of insecticide resistance and understanding the involved mechanisms requires a maintained sampling effort. Collection of live adults is difficult and represents the main barrier to monitoring, so developing effective methods with larvae could enable an increase in data acquisition.

The Yonne area has to be carefully watched to follow the insecticide resistance evolution in pest populations. A knowledge of the resistance level and involved mechanisms are necessary to properly advise farmers; farmers must be advised to avoid useless treatments in areas where pyrethroid efficiency is null. The efficacy of treatments could be prolonged in areas with no fixed resistant populations of pests through a decrease in the use of the pyrethroid insecticide family. Finally, understanding of the involved mechanisms must lead only known-effective treatments being carried out and the provision of effective alternation strategy.

Currently, there are few chemical alternatives to pyrethroids available. In France, in the short term, only organophosphates (alone or in association with pyrethroids) can be used in substitute of pyrethroids for these two pests and the number of applications is limited by legislation. However, agronomic practices – for example: an early sowing date, fertilization, cultivation in association with a frost sensitive legume crop - can significantly reduce cabbage stem flea beetle or rape winter stem weevil attacks or their harmfulness (Cadoux *et al.*, 2015; Terres Inovia.,2017). The integration of all kinds of tactics (agronomical, landscape design, biocontrol, chemical solutions...) will allow farmers to produce oilseed rape in a sustainable way for their farm and the environment.

## **Acknowledgements**

We acknowledge the AFPP (French Association for Plant Protection), the IRAC (Insecticide Resistance Action Committee), the INRA (French National institute for Agricultural Research) Avignon, the co-financers and all the sample collectors (stakeholders, phytosanitary firms, the “Réseau de Surveillance Biologique du Territoire”, agricultural education, the Ecoentomology Laboratory, colleagues from Terres Inovia...), and farmers for allowing us collect insects in their fields.

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## The cabbage stem flea beetle combines several different strategies to overcome the chemical defence in oilseed rape

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**Abstract:** Crucifer-feeding herbivores encounter a potent chemical defense consisting of two components, the  $\beta$ -thioglucoside hydrolase enzyme myrosinase and its glucosinolate substrate (Halkier & Gershenzon, 2006). While glucosinolates themselves are non-toxic, their hydrolysis by the myrosinase enzyme liberates noxious isothiocyanates (ITCs) with strong reactivity towards thiol- and amine-groups in peptides and proteins. Because glucosinolates and myrosinases are stored separately in plants, ITCs are rapidly released in damaged tissue upon herbivory, and their ingestion negatively affects insect growth, development, and survival (e. g. Jeschke *et al.*, 2017; Li *et al.*, 2000). Nevertheless, a number of insect herbivores attack economically important crucifer crops such as oilseed rape, cabbage, and mustard. Among these, the cabbage stem flea beetle *Psylliodes chrysocephala* is one of the most important pests of winter oilseed rape. Previous studies showed that the host plant range of *P. chrysocephala* is restricted to glucosinolate-containing plants, and that glucosinolates stimulate adult feeding (Bartlett *et al.*, 1994; Bartlett & Williams, 1991). These findings demonstrate that *P. chrysocephala* is highly adapted to crucifers, but how this specialist copes with the glucosinolate-myrosinase defense system and avoids ITC toxicity is so far unknown.

Several crucifer-feeding insects are able to prevent the activation of ingested glucosinolates to toxic ITCs by sequestering glucosinolates in their bodies (e.g. Beran *et al.*, 2014; Müller *et al.*, 2001), or by converting them to desulfo-glucosinolates by using a specific glucosinolate sulfatase enzyme (Ratzka *et al.*, 2002). For example, the flea beetle *Phyllotreta striolata* selectively sequesters host plant glucosinolates to up to 2% of its body weight, and in addition produces its own insect myrosinase (Beran *et al.*, 2014).

We analyzed body and faeces extracts of *P. chrysocephala* adults by high performance liquid chromatography, and found that intact glucosinolates were present in the cabbage stem flea beetle as well. Glucosinolates were not only present in adults, but in all life stages including eggs. The total glucosinolate concentration in *P. chrysocephala* was similar to that in its host plant *Brassica rapa* (~ 4  $\mu\text{mol}$  per g fresh weight), and thus much lower than in *P. striolata* (~ 50  $\mu\text{mol}$  per g fresh weight; Beran *et al.*, 2014). We did not detect endogenous myrosinase activity in *P. chrysocephala*, but surprisingly we found desulfo-glucosinolates, showing that this specialist partially detoxifies ingested glucosinolates by desulfation.

To assert whether the cabbage stem flea beetle can prevent glucosinolate hydrolysis in ingested plant tissue by glucosinolate sequestration and desulfation, we analyzed the metabolic fate of 4-methylsulfinylbutyl (4MSOB) glucosinolate in adults in a feeding experiment. Therefore, adults were fed with detached leaves of *Arabidopsis thaliana* spiked with 4MSOB glucosinolate as described in Schramm *et al.* (2012). Afterwards, we extracted adults and faeces, and quantified the amounts of intact and desulfo-4MSOB glucosinolate in these samples by liquid chromatography-tandem mass spectrometry. The total amount of ingested glucosinolate was determined by quantifying the remaining glucosinolates in fed leaves.

In this quantitative study, intact and desulfo-glucosinolates together accounted for only 26% of the total ingested glucosinolate, suggesting that most glucosinolates are nevertheless activated by the plant myrosinase. The presence of glucosinolate hydrolysis products in faeces extracts supported this hypothesis. *Psylliodes chrysocephala* partially detoxified 4MSOB-ITC via the widely conserved mercapturic acid pathway, *i.e.* the conjugation of electrophilic toxins to glutathione, followed by the stepwise hydrolysis to the corresponding cysteinylglycine- and cysteine-conjugates. This common detoxification pathway plays a key role in ITC metabolism in several other lepidopteran and dipteran insects (Gloss *et al.*, 2014; Schramm *et al.*, 2012), but we additionally identified two previously unknown cyclic metabolites derived from the ITC-cysteine conjugate in faeces extracts, demonstrating that ITC metabolism in *P. chrysocephala* differs from that in other insects.

Together, our results revealed a remarkably complex set of adaptations that enable the cabbage stem flea beetle to overcome the characteristic chemical defense in their host plants, and thus contribute to the success of this major oilseed rape pest.

**Key words:** cabbage stem flea beetle, glucosinolate, chemical defense

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**Entomology Session 4:  
Effects of insecticides on pests of OSR  
and their natural enemies**





## Situation regarding important insect pests in oilseed rape in the Czech Republic after four years without neonicotinoid insecticidal seed treatments

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**Abstract:** Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ), as an independent Czech state institution, provide information on the large-scale field monitoring. Of the proportion of land sown to oilseed rape is almost 16% of arable land in the Czech Republic. Higher proportions of oilseed rape in the crop rotation bring problems with increased pests and disease incidence in the crops. Problems with insect pests became noticeably worse after the ban on neonicotinoid seed treatments. Among the most important insect pests of Czech oilseed rape are *Ceuthorhynchus pallidactylus*, *C. napi*, *Brassicogethes aeneus*, and *Dasineura brassicae* with *C. obstrictus* (syn. *assimilis*). Autumn pests became more important a few years before the neonicotinoids ban, especially in the case of *Psylliodes chrysocephala* (= *chrysocephalus*) and generally-occurring *Phyllotreta* spp. After the ban, the pest problems grew. Two years ago, problems with minor pests, e. g. aphids (*Brevicoryne brassicae*, *Myzus persicae*), and *Plutella xylostella* started. In the recent years, the outcomes of state monitoring confirm strong occurrences of oilseed rape pests and consequently severe damages in the crops. The reason for the increasing severity in ‘minority pests’ is not only the changing climate but also the high proportion of oilseed rape in the crop rotation and onset of resistance in intensively-treated crops. The reason for numerous autumn spray applications is the prohibition of seed treatments. The price of oilseed rape is currently stagnating and therefore it is necessary to think about the economy of the crop, which is unprofitable due to the prohibition of seed treatments.

**Key words:** large-scale monitoring, neonicotinoids, resistance, stem weevils, Turnip Yellow Virus

### Introduction

Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ), as an independent state institution, provide information on the large-scale field monitoring. Oilseed rape (OSR) is the second most widely-grown arable crop after cereals (almost 16% of sown area in the Czech Republic). A higher proportion of OSR in the crop rotation brings associated problems including increased prevalence of pests and diseases in the crops. Problems with insect pests became worse after ban on neonicotinoid insecticide seed treatments. Among the most important insect pests of Czech OSR include *Ceuthorhynchus pallidactylus*, *C. napi*, *Brassicogethes aeneus*, and *Dasineura brassicae* along with *C. obstrictus* (= *assimilis*). Autumn pests became more important a few years before the neonicotinoid seed treatment ban, especially in case of *Psylliodes chrysocephalus* and generally-occurring *Phyllotreta* spp.

After the ban, the importance increased. Two years ago, problems with minor pests eg. aphids (*Brevicoryne brassicae*, *Myzus persicae*), and *Plutella xylostella* started.

## Material and methods

### *Data collection*

Data from large-scale field monitoring carried out by the crop inspectors of ÚKZÚZ were used. Data are collected as part of regular crop monitoring checks. Monitoring is carried out by 75 inspectors, who regularly check approximately 100 permanent observation plots, so they know the history of the plot including pesticide inputs. There are 1-2 visits per week during the growing season. Inspectors use visual evaluation as well as a variety of monitoring tools, including optical and pheromone traps, light traps, sucking traps, etc. On average, 60,000 observations are carried out per year. Observations are made both in autumn and spring up to the end of flowering (growth stage BBCH 69).

### *Scope of monitoring*

Among the autumn pests monitored included *Psylliodes chrysocephala*, *Phyllotreta* spp., aphids (*Brevicoryne brassicae*, *Myzus persicae*), and *Plutella xylostella*. Spring pests monitored included *Ceuthorrhynchus pallidactylus*, *C. napi*, *Brassicogethes aeneus* and *Dasineura brassicae* with *C. obstructus*. During the season, monitored diseases included *Phoma lingam* + *L. biglobosa*, *Sclerotinia sclerotiorum* and *Erysiphe cruciferarum*, *Botrytis cinerea* and *Peronospora parasitica*.

## Results and discussion

### *Minor insect pests in OSR*

Two years ago, problems with minor pests, e. g. aphids (*Brevicoryne brassicae*, *Myzus persicae*), and *Plutella xylostella* started. This phenomenon is connected to climate change i.e. the extremely hot summers occurring in previous years. Season 2016/2017 will never be forgotten for the extreme occurrence of Turnip Yellow Virus (TuYV) due to the high infestation of crops by aphids (Figure 1.). Moreover, most of the pesticide application did not work (not even multiple applications of different combination of systemic insecticides); up to 30% of crops had to be resown.

### *Spring pests of OSR*

Regarding spring pests, due to the climate change bringing increasing temperatures, the beginning of the season shifted a month in advance and the first harmful occurrences of stem weevils were detected already in mid-March (in 2015, first recommendation for treatment was reported in mid-April). Another interesting point is the fact that stem weevils often do not appear in yellow bowls and the first recommendation for treatment, thanks to this detail, was delayed by three weeks. Since 2017, the inspectors of ÚKZÚZ have been instructed to monitor stem weevils on plants with higher priority. Half of cases confirmed that weevils occurred with high density causing severe damage on plants while yellow bowls contained individuals hardly near threshold (Figures 2 and 3).

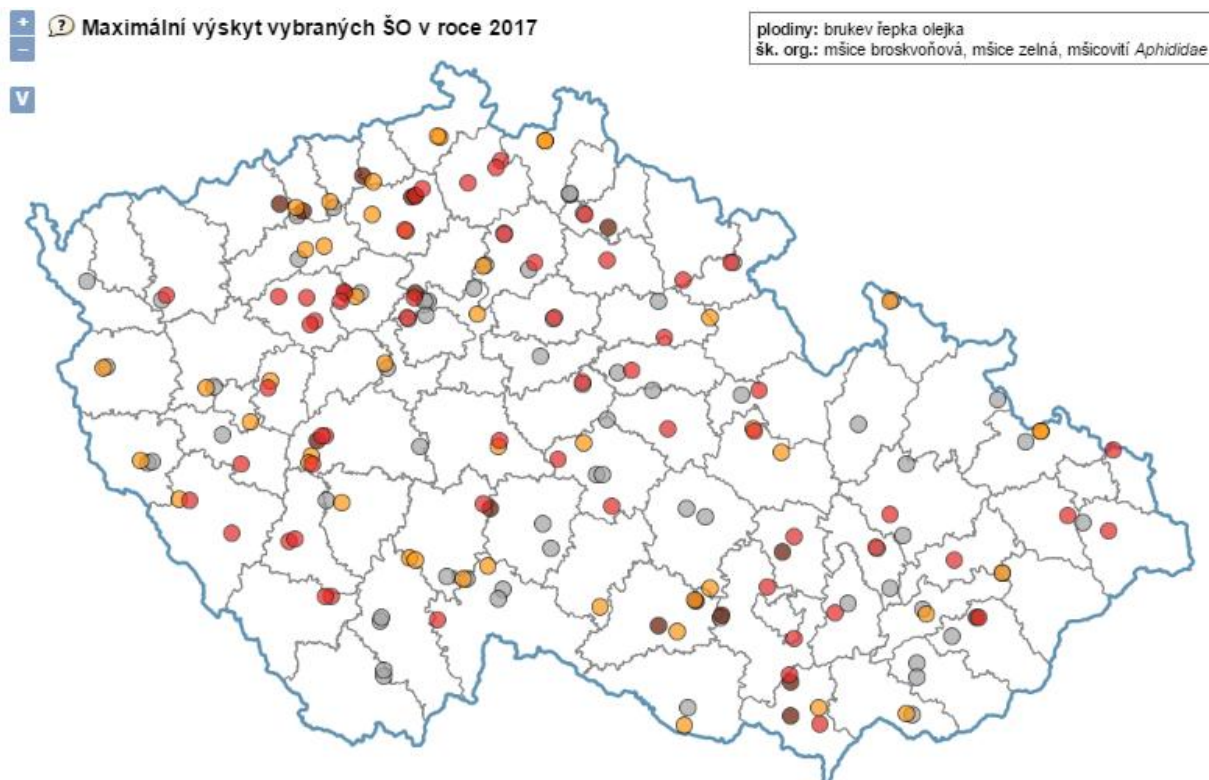


Figure 1. Map showing distribution and abundance of Aphids in oilseed rape crops, Czech Republic, autumn 2017 regarding to TuYV infection (red = harmful, yellow = weak, grey = no occurrence, dark red = occurrence)

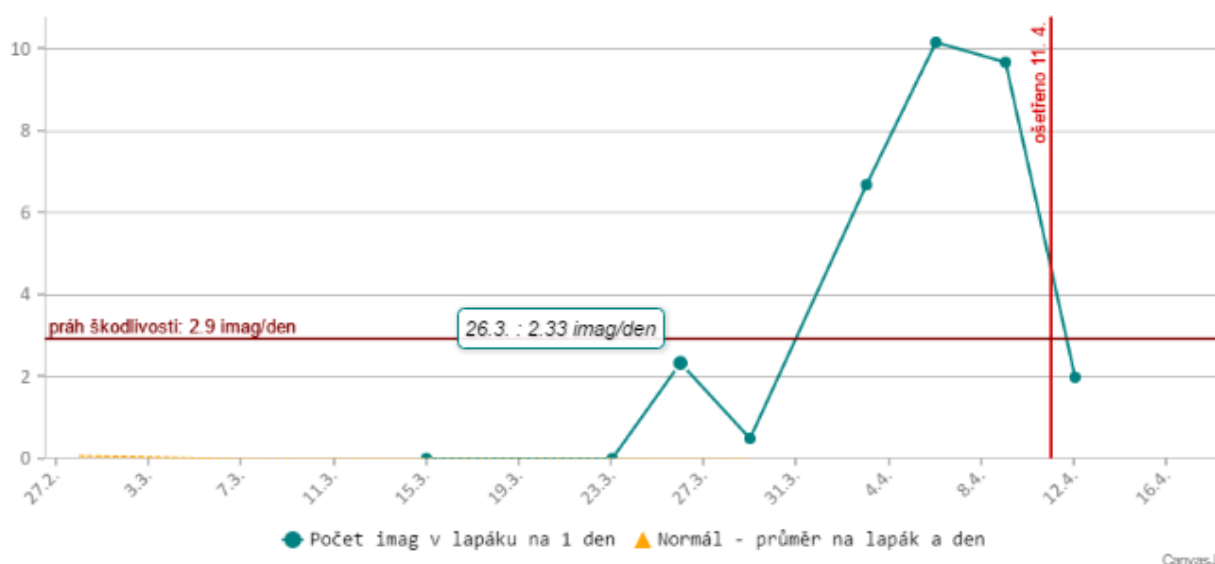


Figure 2. Catches of stem weevils in yellow bowl traps in selected Czech district; blue curve shows numbers of weevils in time, horizontal red line = threshold, vertical red line = date of insecticide application).

First occurrence exceeding threshold confirmed 26.3.2018; the same date, economical damage was observed, see Figure 3.

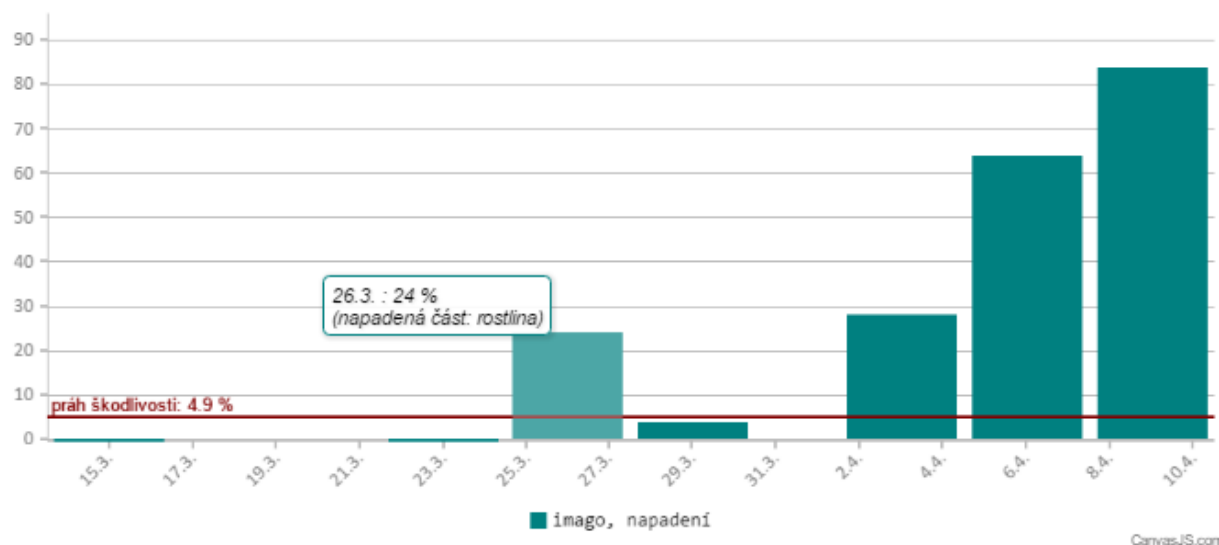


Figure 3. Percentage of damaged OSR plants by stem weevils in selected Czech districts (first damaged plants exceeding treatment threshold confirmed 26.3.2018, while catches of weevils in yellow bowls from the same spot are under the threshold, reference to Figure 2).

## Conclusion

In the recent years, the outcomes of state monitoring confirm strong occurrences of OSR pests and consequently severe crop damage. The reasons for the growing importance of ‘minority’ pests is not only changing climate, but possibly also the high proportion of OSR in the crop rotation and onset of insecticide resistance in populations in intensively treated fields. Usually, three insecticide applications against Cabbage stem flea beetle and Flea beetles are carried out in the autumn, and in spring time, two or three additional applications against stem and pod pests. The reason for numerous autumn applications is the prohibition of seed treatments – statistics on consumption of plant protection products confirm this. The price of OSR is currently stagnating and therefore it is necessary to think about the economy of the crop, which is currently unprofitable due to the prohibition of seed treatments.

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## Efficacy of alternative seed coatings against autumn insect pests of winter oilseed rape (*Brassica napus* L.)

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**Abstract:** In laboratory bioassays, we tested the efficacy of alternative seed coatings against the cabbage stem flea beetle (*Psylliodes chrysocephala* L.) and the cabbage root fly (*Delia radicum* L.), which both attack young winter oilseed rape plants in autumn. Overall, we screened 12 botanicals, seven biologicals and three promoters for their efficacy against adult cabbage stem flea beetles feeding on plants in the cotyledon stage (BBCH 10) and against cabbage root fly larvae mining in the root tissue of young plants (BBCH 12-13). The effects of the seed coatings are expected to be either direct, via repellent and/or toxic activities (mainly botanicals) or indirect, via induced changes in plant metabolites (mainly biologicals and promoters). In a non-targeted approach, we therefore analysed the metabolome of seed coated plants infested by adult cabbage stem flea beetles and cabbage root fly larvae to detect biochemical polymorphism and metabolite markers, respectively, associated with a reduction in feeding damage by these insect pests.

The efficacy of alternative seed coatings against feeding by adult cabbage stem flea beetles was assessed 24 hours, 48 hours, 72 hours and seven days post release of 2 beetles per plant at BBCH 10 (cotyledon stage) (no-choice tests). Results documented that single seed coatings may negatively affect adult beetles within the first three days of assessment as well as after 7 days. Strongest effects were observed for one promoter, which reduced the feeding by adult beetles by about 50% compared with an untreated control with a concomitant high level of variability ranging from 0% (no feeding) to 100% (cotyledons completely consumed).

The efficacy of alternative seed coatings against feeding by cabbage root fly larvae was assessed four weeks post inoculation of five eggs per plant at BBCH 12/13 (no-choice tests). In two subsequent bioassays, we found no or only moderate effects of single seed coatings on the root damage by larvae.

Additionally, we screened for direct repellent and/or toxic activities of single compounds and therefore used the formulated botanicals by proceeding with a similar experimental design for each of the insect pests. With regard to adult cabbage stem flea beetle, the botanicals (different dilutions) were directly applied to the surface of the cotyledons and on a filter paper placed on the soil substrate around the seedlings (BBCH 10), respectively. None of the botanicals reduced the feeding damage by adult beetles when applied onto the cotyledons, whereas strong repellent effects of some compounds were observed when applied on the filter paper. Ongoing bioassays refer to potential repellent and/or toxic effects of formulated botanicals on cabbage root fly larvae via soil drenching (different dilutions) of young plants at BBCH 12/13.

**Key words:** winter oilseed rape, autumn insect pests, seed coatings



## Does the assessment of eggs in *Ceutorhynchus pallidactylus* and *C. napi* ovaries in spring make possible a date for one common spray treatment effective against the weevils and even pollen beetles?

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**Abstract:** This study aims to improve timing of foliar insecticides against cabbage stem (*Ceutorhynchus pallidactylus*) and rape stem (*C. napi*) weevils at localities where *C. pallidactylus* usually highly predominates in relation to *C. napi* during spring. We also aim to address the question whether the stem mining weevils and pollen beetles could be controlled with one spring application when the spray timing is based on proper monitoring of the pests in oilseed rape crops. In the case when only one spring application was used, pyrethroids showed better results in decreasing the levels of stem damage induced by the larvae when they were applied earlier (first females prepared for oviposition). Conversely the combination of organophosphate + pyrethroid is better applied later (when more than 50% females are prepared for oviposition). The use of a combination of organophosphate and pyrethroid makes the delay in the date of first spring spraying more well-founded and it is more suitable when a farmer plans to combine control of stem weevils with control of pollen beetles. So, not only the detailed monitoring of flight activity and the subsequent dissecting of stem weevil females but also the choice of insecticide with substantially longer residual activity is important for the possibility to delay the first spring spray to later dates (and later growth stages).

**Key words:** stem weevils, pollen beetles, winter oilseed rape, Integrated Pest Management, decrease in pesticide use

### Introduction

In Europe the cabbage stem weevil *Ceutorhynchus pallidactylus* (Mrsh.) (Coleoptera: Curculionidae), rape stem weevil *Ceutorhynchus napi* Gyllenhal, 1837 (Coleoptera: Curculionidae) and pollen beetle *Brassicogethes aeneus* (Fabricius, 1775) (Coleoptera: Nitidulidae) rank among the most important insect pests of winter oilseed rape in spring time.

The larvae of the both stem mining weevils damage plants affecting stability, nutrient supply and the podding (Broschewitz, 1985, Büchs, 1998; Lerin, 1995). Damage to the stems induced by *C. pallidactylus* larvae also facilitates infestation by the fungal diseases, *Phoma lingam* and *Botrytis cinerea* (Šedivý & Kocourek, 1994; Alford *et al.*, 2003). The egg deposition of *C. napi* females can result, in some cases and seasons, but not always (Seidenglanz *et al.*, 2009) in twisting and splitting of stem tissues, followed by distortion and disruption of the growth (Juran *et al.*, 2011). Both species can cause significant yield losses (Kelm & Klukowski, 2000; Alford *et al.*, 2003).

Adult pollen beetles damage oilseed rape during the green to yellow bud stages (Šedivý, 1993). They can cause severe damage and yield loss (Ruther & Thiemann, 1997; Alford *et al.*, 2003). In extreme cases an infestation with pollen beetles can cause yield loss of up to 50% (Kirch, 2006). Importance of pollen beetles has been increased recently in many European countries due to populations resistance to pyrethroids. Pyrethroid resistance of pollen beetles is steadily spreading and has continued to increase in Europe since 2005 (Heimbach & Müller, 2013; situation in the Czech Republic described in Seidenglanz *et al.*, 2015 a, b).

On the basis of field trials made and published by Büchs (1998), it is expected, that about 9-11 days after the first flight activity of *C. napi* adults (= adults caught in yellow water traps located in winter oilseed rape), approximately 50% of the females in crop will be prepared for oviposition (= will be carrying mature eggs in their ovaries). *Ceutorynchus pallidactylus* differs in this. It was experimentally confirmed that the male and female cabbage stem weevils leave their hibernation sites at distinctly different times (Büchs, 1998; Klukowski, 2006). Females are delayed in leaving the hibernation sites and as a consequence, migrate to crops later than males. At the beginning of flight activity, proportions of males in yellow water traps are markedly higher than females. The sex ratio in adults of *C. pallidactylus* caught in yellow water traps and also present in crop equalizes in the course of time (Büchs, 1998; Klukowski, 2006; Seidenglanz *et al.*, 2009). This limits the possibilities of copulation at the beginning of immigration to crops. So, the higher proportions of *C. pallidactylus* females carrying mature eggs is possible to record in crops markedly later than in the case of *C. napi* populations present in the same crops. According to Büchs (1998) and Seidenglanz *et al.* (2009) it is expected that about 28 days after the record of the first flight activity of *C. pallidactylus* adults in winter oilseed rape crops (= first adults caught in yellow water traps), approximately 50% of females will be prepared for oviposition. So, the process takes almost three weeks more in *C. pallidactylus* than in *C. napi* populations. The time when higher portions of *C. pallidactylus* females carrying mature eggs appear in crops often coincides with the first flight activity peaks of *B. aeneus*.

This evokes the question of whether it would be possible to control stem mining weevils and pollen beetles with only one insecticidal application, at least in the sites or in the seasons when *C. napi* abundance is low. Regardless of the fact that the regions, where (and seasons when) *C. napi* abundance is low and *C. pallidactylus* highly predominates are not so rare in Europe (situation in the Czech Republic described by Seidenglanz *et al.*, 2013), farmers regularly apply two consecutive insecticide sprays every spring: the first one against stem weevils in March/April, and the second one primarily against pollen beetles in the second part of April or at the beginning of May. Growers even advisors mostly perceive stem weevils and pollen beetles as two, temporally separated problems which need different approaches to be successfully controlled (Kocourek *et al.*, 2017).

This study aims to improve timing of insecticide applications against cabbage stem (*C. pallidactylus*) and rape stem (*C. napi*) weevils at the localities where *C. pallidactylus* usually highly predominates to *C. napi* during the spring. At the same time, we try to address whether stem mining weevils and pollen beetles could be controlled with one spring application at such places when the spray timing is timed according to proper monitoring of the pests in crops. We compared effects of two types of insecticides with different duration of residual activity and modes of action (pyrethroid vs. pyrethroid + organophosphate) applied on different dates (according to results of dissecting of *C. pallidactylus* and *C. napi* females caught in yellow water traps) on levels of damage induced by the weevil larvae and at the same time on levels of damage caused by pollen beetles (*B. aeneus*) on the main racemes. The importance and potential contribution of another spring application (primarily aimed at pollen beetles) in reducing the damage caused by the both pests was also assessed.

## Material and methods

Small (25 m<sup>2</sup>) plot trials comprising 10 treatments replicated four times were carried out in 2016, 2017 and 2018. The trials were located near Šumperk (North-eastern part of the Czech Republic, 49.9815608N, 16.9999725E) in all three years. *Ceutorhynchus pallidactylus* usually highly predominates with respect to *C. napi* in this region in spring (March/April, April, May) (Seidenglanz *et al.*, 2013; Kocourek *et al.*, 2017). Winter oilseed rape cv. Orava was used. Three yellow water traps were placed in buffer zones of the trial (= untreated crop immediately surrounding the trial) every year. The traps were emptied twice per week. For each of the sampling dates the following assessments were recorded: number of adult of *C. pallidactylus* and *C. napi*, number of males and females of the two species, and the number of the females of both species with ripe eggs in their ovaries. Adult pollen beetles (*B. aeneus*) were also counted. The dates of spraying were timed according to results of the female weevil dissections (Seidenglanz *et al.*, 2013): Insecticides (pyrethroid vs. pyrethroid + organophosphate, Table 1) were applied on two different dates (timing I and II). Timing I = the date when the first females with ripe eggs in their ovaries were recorded from the yellow water traps; timing II = the date when the majority (more than 50%) of the females caught in the traps had ripe eggs in their ovaries. The length of the time period between timing I and II was at least 7 days (Table 2). Each of the treatments (each of the insecticides applied in timing I or II) was either followed with another insecticide application (timing III) or not (no further application after II). Timing III = spraying primarily aimed against pollen beetles: pymetrozine in 2016, 2017; indoxacarb in 2018; Tables 1, 2).

Table 1. The list of treatments applied to oilseed crops (Czech Republic, 2016, 2017 and 2018).

treatment no.	treatment description <sup>1</sup>	application against weevils (timing I or II)	application against pollen beetles (timing III)
1	untreated control I	no	no
2	25 g cypermethrin / ha (57.5 g etofenprox /ha)	I	
3	25 g cypermethrin / ha (57.5 g etofenprox /ha)	II	
4	30 g cypermethrin / ha + 300 g chlorpyrifos-ethyl / ha	I	
5	30 g cypermethrin / ha + 300 g chlorpyrifos-ethyl / ha	II	
6	25 g cypermethrin / ha (57.5 g etofenprox /ha)	I	yes (75 g pymetrozine/ha in 2016, 2017; 25.5 g indoxacarb/ha in 2018)
7	25 g cypermethrin / ha (57.5 g etofenprox /ha)	II	
8	30 g cypermethrin / ha + 300 g chlorpyrifos-ethyl / ha	I	
9	30 g cypermethrin / ha + 300 g chlorpyrifos-ethyl / ha	II	
10	untreated control II	no	

<sup>1</sup>pyrethroid in tr. 2, 3, 6 and 7 = cypermethrin applied in 2016 and 2017; etofenprox in 2018



Table 2. The dates of insecticide application in relation to the proportions of female weevils prepared for oviposition (i.e. with ripe eggs in their ovaries) and some results of flight activity monitoring (for seasons 2016-2018).

season	information about crop and <i>C. pallidactylus</i> and <i>C. napi</i> at the dates of spraying:					max. flight activity in the season (species and sex: date, number per trap per 3 days) <sup>3</sup>	flight activity lengths for weevils and pollen beetles <sup>4</sup>	length of egg-laying period (for <i>C. pallidactylus</i> and <i>C. napi</i> )	First record of $\geq 9$ adults of <i>C. pallidactylus</i> ( <i>C. napi</i> ) / yellow trap within 3 days <sup>5</sup>
	date (timing) <sup>1</sup>	crop growth stage	% of females with ripe eggs (both species) <sup>2</sup>	mean number of <i>C. napi</i> females / trap / 3 days	mean number of <i>C. pallidactylus</i> females / trap / 3 days				
2016	I: 1.4.	BBCH 31, 50; height 12 cm	7.69	0.25	3.25	<i>C. pall.</i> males: 8.4., 35.67; <i>C. pall.</i> females: 8.4., 9.00; <i>B. aeneus</i> : 15.4., 299.45	<i>C. pall.</i> : 14.3.-1.6.; <i>C. napi</i> : 17.3.-14.4.; <i>B. aeneus</i> : from 30.3.	<i>C. pall.</i> : 1.4.-10.5.; <i>C. napi</i> : 1.4.-20.4.	23.3.
	II: 8.4.	BBCH 53; height 40 cm	50.45	0.00	9.00				
	III: 22.4.	BBCH 55; height 95 cm	71.35	0.00	0.67				
2017	I: 31.3.	BBCH 31; height 10 cm	26.00	0.78	3.42	<i>C. pall.</i> males: 3.4., 45.33; <i>C. pall.</i> females: 3.4., 12.33; <i>B. aeneus</i> : 15.5., 258.46	<i>C. pall.</i> : 16.3.-11.6.; <i>C. napi</i> : 2.3.-26.5.; <i>B. aeneus</i> : from 30.3.	<i>C. pall.</i> : 28.3.-5.6.; <i>C. napi</i> : 28.3.-22.5.	28.3.
	II: 10.4.	BBCH 53-55; height 45 cm	86.73	0.96	8.08				
	III: 3.5.	BBCH 57-59; height 100 cm	100.00	0.16	3.76				
2018	I: 12.4.	BBCH 50; height 10 cm	26.67	0.00	5	<i>C. pall.</i> males: 6.4., 116.33; <i>C. pall.</i> females: 25.4., 11.67; <i>C. napi</i> males: 6.4., 4.33; <i>C. napi</i> females: 6.4., 5.00; <i>B. aeneus</i> : 18.5., 21.33	<i>C. pall.</i> : 29.3.-30.5.; <i>C. napi</i> : 29.3.-25.4.; <i>B. aeneus</i> : from 6.4.	<i>C. pall.</i> : 9.4.-30.5.; <i>C. napi</i> : 9.4.-25.4.	3.4.
	II: 19.4.	BBCH 51-53; height 15 cm	73.91	1.33	7.33				
	III: 26.4.	BBCH 53-55; height 45 cm	86.49	1.33	11.67				

<sup>1</sup>date III = the date when insecticide applications aimed at pollen beetles was made (only in treatments 6-10)

<sup>2</sup>both species = females of *C. pallidactylus* and *C. napi* assessed together as one group

<sup>3</sup>numbers of *C. napi* are not included in the column for 2016 and 2017 as their numbers did not exceed the value of 3 adults per trap per 3 days in the two seasons

<sup>4</sup>for pollen beetles only the start of flight activities are recorded in the table – they were present in crops to the first half of June

<sup>5</sup>common European threshold (also used in Czech Republic) of  $\geq 9$  adult individuals of *C. pallidactylus* (*C. napi*)/yellow trap within 3 days

Apart from the described tested applications, two other sprays were made to all plots in the trials during the crop vegetation – maintenance applications with herbicides before winter: 1) preemergent spraying with metazachlor + clomazone against dicotyledons; 2) postemergent spraying with quizalofop-P-ethyl monocotyledons.

Total lengths of flight activity stated for the weevils and pollen beetles are the periods between the date of their first presence in the yellow water traps and the last presence in the traps in the individual years (2016, 2017, 2018) (Table 2).

Total lengths of egg-laying periods stated for the weevils are the periods between the date when the first female with ripe eggs in her ovaries was detected from the yellow water traps and the date when the last one was present in the traps in the individual years (2016, 2017, 2018) (Table 2).

The levels of plant damage caused by weevil larvae and pollen beetle adults were assessed when the crop achieved green maturity stage (14.6. 2016, 9.6. 2017, 11.6.2018). On those dates only negligible portions of the most delayed weevil's larvae (only *C. pallidactylus*) still occurred in plants; the majority had already left the plants to pupate in soil. We therefore regard the levels of stem damage caused by the larvae on those dates to be final. At the same time it was already possible to count final numbers of green pods on the main racemes. Twenty plants from every plot (n per treatment = 80) were randomly selected and for each of them we assessed: 1) the length of damage induced by the *C. pallidactylus* and *C. napi* larvae per stem and 2) the number of pods evolved on the main raceme (pollen beetles damage buds which may cause decrease in pod numbers; on the other hand the number of pods can be decreased also due to the weevil's larvae presence in stems).

When the crop achieved full maturity the individual plots were separately harvested using a small plot harvester (Wintersteiger Quantum). The plot yields (after cleaning and drying) were then weighed and recorded data statistically analyzed (n per treatment = 4).

The results of the trials were analyzed using Statistica version 12 software. For all sets of data (levels of stem damage caused by the weevil larvae, levels of total stem damage, numbers of pods on main racemes and plot yields), a one-way ANOVA was used with LSD for variance analysis, followed by Tukey tests for multiple comparisons between treatments ( $P < 0.05$ ). For the ANOVA, the homogeneity of variance was previously checked using Bartlett tests ( $P < 0.05$ ).

## Results

The locality selected for the trials has been characteristic with high predominance of *C. pallidactylus* with respect to *C. napi* in the course of spring. Numbers of *C. napi* adults caught in yellow water traps did not exceed the treatment threshold value of 3 specimens per trap per 3 days in 2016 and 2017. Only in 2018 were their numbers somewhat higher. In contrast, the numbers of *C. pallidactylus* adults exceeded common thresholds in each of the three years. The numbers of males of the species recorded in the yellow water traps at the time of maximal flight activity varied among the individual seasons much more (35.67 males/trap/3 days in 2016 – 116.33 males/trap/3 days in 2018) than the numbers of females (9.00, 12.33, 11.67 females/trap/3 days in 2016, 2017, 2018). Only in one season did the first *C. napi* adults appear in the yellow water traps markedly earlier than the first *C. pallidactylus* migrants (2017). In two seasons (2016, 2018) the total lengths of flight activity periods of *C. pallidactylus* were markedly longer than the flight periods of *C. napi* at the locality. In all three years the flight activity of *C. pallidactylus* lasted at least to the end of May. First females with ripe eggs in the ovaries appeared in the traps from the end of March to the first third of April. In all three years the females of *C. pallidactylus* were still able to lay eggs during May. In 2018 they prolonged oviposition until June. The oviposition periods for *C. pallidactylus* were markedly longer than for *C. napi* in 2016 (2.5 months vs. 1 month) and 2018 (2 months vs. 1 month) (Table 2). In 2016 and 2017 the first pollen beetles appeared in yellow water traps relatively early (30.3., BBCH 31). In 2018 the first migrants arrived on April 6 (BBCH 31 – 50) (Table 2).

In the course of 2018, the numbers of pollen beetles present on racemes (counted on tr. 1) did not exceed the Czech thresholds (1-3 pollen beetles per inflorescence according to the growth stage; Kocourek *et al.*, 2017). The numbers of adults caught in yellow water traps were also low in this season. There were markedly higher numbers of pollen beetles in the yellow water traps in 2016 and 2017; in 2016 the abundance rapidly increased after April 10. The threshold was exceeded on April 12, maximal flight activity was recorded on April 15. Relatively high levels of infestation (approximately 4-7 adults per inflorescence on plots of tr. 1 control) remained until the end of April (between the growth stages BBCH 53 and BBCH 59). Even though the numbers of beetles caught in yellow water traps at the time of maximal flight activity were similar in 2016 and 2017 (300 and 259 adults/trap/3 days, respectively) the situation was different in the two seasons. Due to unusually cold weather in the course of April 2017 the abundance of flight activity of pollen beetles was low at the time (BBCH 33-BBCH 59). The abundance increased slightly above the threshold for the first at the beginning of May (BBCH 57-59). The highest numbers of adults on inflorescences (and the highest level of flight activity, too) were recorded when the plants were already in flower (15.5. 2017; BBCH 63-65).

The levels of stem damage induced by weevil's larvae are listed in Table 3. In 2016 all the treatments in which the control of stem weevils was made (tr. 2-9) showed significantly less stem damage than the control. Cypermethrin applied solo (tr. 2, 3, 6 and 7) showed better results when the spraying was made at timing I (01.04.2016: 7.69% of females with ripe eggs) regardless of whether the next application (at timing III) was made or not. In contrast spraying with cypermethrin + chlorpyrifos-ethyl (tr. 4, 5, 8 and 9) showed better results when the application was made at timing II (08.04.2016: 50.45 % of females with ripe eggs). The next spray (made at timing III) markedly contributed to the increase of the effect of the insecticide treatments (compare tr. 5 and 9). In 2017 there were smaller differences in the levels of damage among the individual treatments in comparison with results recorded in 2016 and 2018. Cypermethrin applied solo at timing II (08.04.2017: 86.73 % of females with ripe eggs) did not result in a significant decrease in the level of stem damage caused by larvae when the next spray (at timing III) did not follow (tr. 3). When the next application followed (tr. 7) the level of stem damage markedly decreased. In 2018 only the treatments 2, 5, 7 and 9 showed significantly less stem damage in comparison with both controls (tr. 1 and 10). In the case of pyrethroids applied solo (etofenprox in 2018) that was more suitable to spray at timing I (12.04.2018: 26.67% of females with ripe eggs), when the next spraying (timing III) did not follow. When the next spraying (timing III) followed, the application made at timing II (19.04.2018: 73.91% of females with ripe eggs) proved to be more effective. In the case of a combination of cypermethrin and chlorpyrifos-ethyl, the sprays made at timing II proved to be more effective. In all three years the application made only at timing III (tr. 10) had a negligible effect on the final level of stem damage. On the other hand, the applications markedly contributed to better effects of some sprays made at timing II – especially in the case of the combination of cypermethrin and chlorpyrifos-ethyl.

The numbers of pods developed on the main racemes at the stage of green maturity are listed in Table 4. No significant differences among the treatments were recorded in 2016. In 2017, plants from tr. 7 and 9 had significantly more pods on main racemes than plants from tr. 1 control. In 2018, not only tr. 7 and 9 but also tr. 5 showed significantly more pods (again in comparison with tr. 1 control). None of the applications made either at timing I or II (and also regardless of the number of applications, tr. 2-9) resulted in significant increases in pod numbers in comparison with the mean recorded for tr. 10 in any of the three years.

Table 3. Damage of oilseed rape stems (caused mainly by *C. pallidactylus* and partly by *C. napi* weevil larvae) recorded in trials in Czech Republic 2016-2018.

treatment	damage of stems induced by <i>C. pallidactylus</i> and <i>C. napi</i> larvae								
	2016			2017			2018		
	mean length of stem damage (cm) <sup>1</sup>	SD	95 % CL (cm)	mean length of stem damage (cm)	SD	95 % CL (cm)	mean length of stem damage (cm)	SD	95 % CL (cm)
1	30.97a	14.69	27.70-34.24	21.70a	13.50	18.70-24.70	30.83ab	14.15	27.18-34.49
2	12.97c	11.61	10.38-15.55	11.24bc	11.68	8.64-13.84	25.22bc	13.59	21.71-27.73
3	18.96b	10.63	16.60-21.33	16.39ab	16.49	12.72-20.06	31.03ab	14.63	27.25-34.81
4	14.29bc	11.57	11.71-16.86	13.31bc	14.55	10.08-16.55	29.49abc	14.60	25.72-33.26
5	12.70c	9.79	10.52-14.88	11.88bc	12.66	9.06-14.69	20.13de	13.65	16.61-23.66
6	13.27c	8.87	11.30-15.25	11.25bc	11.98	8.59-13.92	26.20abcd	11.67	23.19-29.22
7	17.09bc	14.65	13.83-20.35	10.23bc	11.54	7.66-12.79	23.15cde	13.84	19.57-26.73
8	14.45bc	13.04	11.55-17.36	12.19bc	11.92	9.54-14.84	31.35ab	15.48	27.35-35.35
9	7.04d	7.98	5.26-8.81	9.65c	13.79	6.58-12.72	17.05e	14.92	13.20-20.90
10	28.58a	16.02	25.01-32.14	21.40a	15.64	17.92-24.88	32.53a	14.89	28.68-36.37
	F = 29.769; p < 0.001			F = 8.6318; p < 0.001			F = 8.3085; p < 0.001		

<sup>1</sup>the mean values placed in the same column are significantly different when they are marked with different letters

Table 4. Differences in numbers of pods evolved on main racemes of oilseed rape in different treatments of a field experiment, Czech Republic 2016-2018.

treatment	number of pods on main racemes								
	2016			2017			2018		
	mean number of pods per main raceme <sup>1</sup>	SD	95 % CL (cm)	mean number of pods per main raceme <sup>1</sup>	SD	95 % CL (cm)	mean number of pods per main raceme <sup>1</sup>	SD	95 % CL (cm)
1	28.70a	10.37	26.39-31.01	36.20a	11.50	33.64-38.76	27.37a	8.27	25.23-29.50
2	29.34a	7.42	27.69-30.99	39.05ab	10.13	36.80-41.30	28.48a	8.58	26.27-30.10
3	27.53a	7.18	25.93-29.12	38.48ab	11.20	35.98-40.97	31.52abc	8.05	29.44-33.60
4	27.86a	5.47	26.65-29.08	40.75ab	10.71	38.37-43.13	31.58abc	8.22	29.46-33.71
5	30.15a	7.66	28.45-31.85	38.75ab	10.18	36.48-41.02	34.32c	8.38	32.15-36.48
6	29.39a	5.98	28.06-30.72	39.11ab	10.84	36.70-41.52	29.28ab	10.50	26.57-31.99
7	30.78a	7.85	29.03-32.52	41.09b	9.54	38.97-43.21	35.45c	10.85	32.65-38.25
8	30.24a	7.49	28.57-31.90	38.96ab	10.56	36.61-41.31	28.70a	9.52	26.24-31.16
9	38.19b	8.88	36.21-40.16	41.58b	10.76	39.18-43.97	33.82bc	9.01	31.49-36.15
10	30.15a	7.86	28.40-31.90	37.43ab	11.20	34.93-39.92	31.32abc	10.21	28.68-33.95
	F = 11.976; p < 0.001			F = 1.9131; p < 0.05			F = 5.2556; p < 0.001		

<sup>1</sup>the mean values placed in the same column are significantly different when they are marked with different letters

The results of yield assessments are in Table 5. In 2016, tr. 5, 6, 8 and 9 showed significantly higher seed yields than tr. 1 control. Significantly higher yields were achieved from tr. 5, 8 and 9 than from control II (tr. 10). In 2017, tr. 4-9 were significantly more productive in comparison with tr. 1 control. Tr. 5 and 9 were also more productive than control II (tr. 10) in this season. In 2018, tr. 5, 6, 8, 9 and also tr. 10 (control II) were more productive than control I (tr. 1), but none of the compared treatments showed higher yield than tr. 10.

Table 5. Seed yields from plots (plot area = 25 m<sup>2</sup>) under different insecticide treatments, trials in the Czech Republic 2016-2018.

treatment	plot yields								
	2016			2017			2018		
	mean plot yield of seeds (kg/25 m <sup>2</sup> ) <sup>1,2</sup>	SD	95 % CL (cm)	mean plot yield of seeds (kg/25 m <sup>2</sup> ) <sup>1,2</sup>	SD	95 % CL (cm)	mean plot yield of seeds (kg/25 m <sup>2</sup> ) <sup>1,2</sup>	SD	95 % CL (cm)
1	9.87a	0.25	9.48-10.27	9.38a	0.27	8.95-9.81	9.22a	0.55	8.35-10.08
2	10.11ab	0.27	9.68-10.54	9.58ab	0.21	9.25-9.91	9.81a	0.25	9.41-10.20
3	10.28ab	0.25	9.88-10.68	9.93abc	0.18	9.64-10.22	9.92ab	0.29	9.46-10.37
4	10.33ab	0.25	9.94-10.73	10.35cde	0.25	9.96-10.75	9.83a	0.15	9.58-10.07
5	11.86d	0.21	11.53-12.18	11.20ef	0.31	10.71-11.69	10.94c	0.09	10.78-11.09
6	10.83bc	0.23	10.46-11.20	10.73def	0.24	10.35-11.11	10.70c	0.13	10.49-10.91
7	10.45ab	0.23	10.09-10.82	10.26bcd	0.27	9.84-10.68	10.00a	0.14	9.77-10.23
8	11.58cd	0.32	11.08-12.08	10.93def	0.36	10.35-11.50	10.82c	0.14	10.60-11.03
9	12.13d	0.30	11.66-12.60	11.48f	0.32	10.97-11.98	11.16c	0.21	10.82-11.50
10	10.32ab	0.26	9.91-10.73	10.12abcd	0.34	9.58-10.65	10.66bc	0.19	10.36-10.96
	F = 37.956; p < 0.001			F = 24.140; p < 0.001			F = 26.068; p < 0.001		

<sup>1</sup>the plot yields (seeds after cleaning) were corrected according to the moisture content recorded on the date of weighing (standardized on moisture content of 8%)

<sup>2</sup>the mean values placed in the same column are significantly different when they are marked with different letters

## Discussion

For the timing of applications targeted against the stem weevils in this study (timing I and II) we did not follow the common agricultural practice ( $\geq 9$  adult individuals of *C. pallidactylus* or *C. napi*/yellow trap within 3 days) at all (recently for example in Milovac *et al.*, 2017). At the same time we did not use any other (less known) thresholds for the timing of the applications against the pests (Garbe *et al.*, 1996; Prieditis, 1999) – we concentrated exclusively on the females and especially on the developmental stage of their eggs in the ovaries. In this we followed (with some substantial distinctions of course) Büchs's study (1998) and our previous paper (Seidenglanz *et al.*, 2009). The reason for that is that such approach offers a possibility to delay the first spring application to later dates.

Regarding the way of assessment of the levels of stem damage caused by the larvae we decided (in contrast to EPPO guidelines, 2004) to count neither weevil larvae nor the exit holes made by them. In agreement with Milovac *et al.* (2017) we think that the assessment based on the numbers of larvae present in stems recorded at a certain time (in the most cases one assessment is made) would give incomplete results. At the localities where the dominant species is *C. pallidactylus*, such assessment has to led to inaccurate results, especially in the years with lengthy egg-laying periods; at any time during flowering stage only part of the larval population can be recorded in stems, some of them can be present in leaf stalks and some already pupating in the soil.

For the insecticide application timing III, pymetrozine (2016, 2017) or indoxacarb (2018) were selected because they have been recommended for use against pollen beetles – they are highly effective on the pest and also suitable for antiresistance strategies (Heimbach & Müller, 2013) but on the other hand they have shown relatively low effects on stem weevils (Seidenglanz *et al.*, 2018). We intended to use insecticides which would especially effect pollen beetles (and not/only slightly affect the stem weevils) for the application.

The three seasons differed in the levels of the flight activity of the observed insect pests, their abundances in crop, the periods during which they were active (also in relation to the growth stages of crop) and in the levels of damage caused by them. The season 2016 was characteristic with high flight activity of *C. pallidactylus*, negligible counts of *C. napi* adults in yellow water traps, rather shorter length (in comparison with the other two seasons) of *C. pallidactylus* egg-laying period (01.04.-10.05.), high levels of stem damage induced by *C. pallidactylus* larvae and high abundance of *B. aeneus* at the time when plants are highly susceptible to this pest (BBCH 53-55). The season 2017 differed from the previous one in a markedly longer egg-laying period for *C. pallidactylus* (28.03.-05.06.) and a substantially delayed flight activity of *B. aeneus*. The start of their flight activity was shifted to the beginning of May. This resulted in the beetles appearing in high abundance for the first time in in the flowering crop (BBCH 63). Both of the factors (lengthy egg-laying period of *C. pallidactylus* and the delay in the start of *B. aeneus* flight activity) were probably caused by low temperatures during April. The season 2018 differed from the two previous ones in two main features. Somewhat higher counts of *C. napi* were recorded in yellow water traps. So, *C. pallidactylus* (although it still highly predominated) was not the only important stem weevil in this season. Also, the abundance of *B. aeneus* adults remained low in crops during the whole spring.

According to Büchs (1998) it should take about 28 days until approximately 50% of females with ripe eggs are present in traps (and also in the crop) after the first record flight activity. According to our results (2016-2018) the period should be somewhat shorter for *C. pallidactylus*. In 2016 it was 25 days (14.03.-08.04.), in 2017 it was about 20 days (16.03.- approx. 05.04.) and in 2018 it was about 17 days (29.03.- approx. 15.04.), (dates from Table 2). According to Büchs (1998) the first egg-carrying females should be recorded in yellow water traps on average 15 days after the first flight activity. We recorded similar results: the first females with ripe eggs in ovaries (= the date of start of egg-laying period in Table 2) appeared in the yellow water traps after 18 days in 2016, 12 days in 2017 and 11 days in 2018 following the first flight activity.

In the case when only one spring application was used (sprayed at timing I or II; no spray in timing III) pyrethroids (applied solo; tr. 2 and 3) showed better results in decreasing the levels of stem damage caused by the larvae when they were applied earlier (timing II = tr. 2). It proved to be marked in all three years (but statistically different between tr. 2 and 3 in 2016 only). In contrast, the combination of organophosphate + pyrethroid (tr. 4 and 5) should be better be applied later (timing II = tr. 5). The most apparent effect was seen in 2018. So, when

a farmer plans to use a pyrethroid (solo) for treatment against the stem weevils they should not delay the timing too much – it is possible to say that in this case he could follow recent common practice. In this study the timing I (in relation to date of reaching the common threshold) was: 8% of females carrying ripe eggs, 8 days after the reaching of common threshold in 2016, 26% of females carrying ripe eggs and 3 days after the reaching of common threshold in 2017, 27% of females carrying ripe eggs and 9 days after the reaching of common threshold in 2018. The use of a combination of organophosphate and pyrethroid makes the delay in the date of first spring spraying more well-founded and it is more suitable when a farmer plans to combine control of stem weevils with control of pollen beetles. So, not only information on flight activity and the subsequent dissection of stem weevil females is needed but also the selection of an insecticide with substantially longer residual activity is important for the possibility to delay the first spring spray to later dates (and later growth stages).

The contribution of another spring insecticide application (sprayed following timing I or II; and also in timing III) for decreasing the levels of damage induced by the stem weevil larvae proved to be more significant especially in treatments sprayed for the first at timing II (more pronounced in treatments sprayed with the combination of pyrethroids and organophosphate). Regardless of the importance of the second application (timing III) for the control of pollen beetles, the reason for the second spray should also be an impossibility to control the stem weevils successfully with only one spray – especially at the seasons when the egg-laying period is lengthy – due to weather conditions or due to the fact that both species of stem weevils are present in important abundance. The seasons with prolonged egg-laying periods will be more frequent in future as a consequence of climate change (Eickermann *et al.*, 2014). In this study the seasons 2017 and 2018 represent that situation – length of egg-laying periods of stem weevils on one hand and very low importance of pollen beetles on the other.

The search for the one suitable timing for treatment against stem weevils and pollen beetles will be complicated, even if the exact monitoring of the stem weevil activity is used; in many seasons the reason for the impossibility to find such a date will not be the fact of the time separation between the occurrence of the stem weevils and pollen beetles in crops, but the very lengthy egg-laying period of stem weevils. But this is not reason why we should stop to better understand spray timing effects because widespread resistance in pest populations (especially in pollen beetles) and increasing public concern about the environmental hazards of pesticides are threatening the availability of a variety of insecticides. There is therefore a need to avoid any overuse or misuse of insecticides in order to minimise negative effects on environment and the risk of further resistance development (Thieme *et al.*, 2006).

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## Preconditions for pod infestation by brassica pod midge (*Dasineura brassicae*) and control options in oilseed rape

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**Abstract:** Brassica pod midge (*Dasineura brassicae*) can cause severe pod damage in some years and therefore knowledge about its occurrence in the field and necessary conditions for successful oviposition is important. The aim is to achieve sufficient protection of the crop by targeted applications on the one hand and to avoid unnecessary sprays of insecticides on the other hand. Unlike other insect pests of oilseed rape it is difficult for farmers to monitor the midge within the crop and monitoring requires a lot of experience. Weather conditions including temperature (Ankersmith, 1956), wind (Sylvén, 1970) and precipitation during flight period may play an important role for the extent of pod infestation and could be considered in decision support regarding whether a treatment is necessary or not.

Axelsen (1994) studied the development stages of *D. brassicae* under constant temperatures in the laboratory and calculated thermal requirements of the different stages. In our study further experiments were made to determine the oviposition rate of *D. brassicae* at different temperatures. Larvae of the first generation were collected from pods in the field and reared to adult midges in the laboratory (15 °C, 16:8 L:D). After emergence 50 (30) female midges were caged on 20 (12) pods. Each pod was punctured with an insect needle twice to offer suitable oviposition sites for the midges. Pods were placed in climate cabinets at temperatures between 13 °C and 30 °C for 48 h and then dissected and eggs of *D. brassicae* counted.

For control of brassica pod midge, spray applications with the neonicotinoids Biscaya (a. i. thiacloprid) and Mospilan (a. i. acetamiprid) were done from 2016-2018 in JKI field trials in oilseed rape near Braunschweig using conventional spraying equipment as well as with the novel dropleg spraying technique at the flowering stage (BBCH 64-67) of the crop. The timing of the application was focused on the flight period of brassica pod midge, which was determined by the use of photoelectors in the previous years' oilseed rape fields. Efficacy of treatments was evaluated by water trays at soil level catching *D. brassicae* larvae dropping to the ground for pupation. The level of pod infestation was determined by examination of 12 plants per plot after the first and the second generation of larvae finished development, respectively.

Results differ between the years but generally it was found that the dropleg technique could not control pod midge as well as conventional spraying. Conventional application of Biscaya reduced significantly pod infestation in each year, whereas the effect of use of the dropleg technique was not as good as with conventional application but better than the untreated control. Numbers of *D. brassicae* larvae dropping to the ground over the whole vegetation period was reduced with both types of application technique. Level of pod infestation in unsprayed control plots varied between 4% and 30% with the highest infestation rate in 2018. Cabbage seedpod weevil (*Ceutorhynchus obstrictus*) was only abundant in significant numbers in 2018.

**Key words:** brassica pod midge, dropleg technique, insecticides, application

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## **Pyrethroid insecticides in oilseed rape – are they responsible for morphological variations of *Oedothorax apicatus* Blackwall, 1850 (Araneae: Linyphiidae)?**

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**Abstract:** Agrochemicals can have significant influence on the biodiversity of predators in terrestrial agroecosystems. Pesticides in general can have lethal or sublethal effects on spiders and influence their activity, locomotion, web building – and reproduction behaviours as well as abundance. The effect of pesticides on spider morphological variations has been poorly investigated. In this study, we explored the influence of pyrethroid insecticides (Fastac<sup>®</sup>, Talstar<sup>®</sup>, Trebon<sup>®</sup>) on morphological variations in body length, carapace and abdomen length, carapace and abdomen width, and carapace shape in *Oedothorax apicatus* (Blackwall, 1850) collected from conventional, integrated and organically-produced oilseed rape crops. Multiple applications of various pyrethroid insecticides on the conventional field significantly influenced morphological variability in female specimens. These females had longer bodies with longer and wider carapace and abdomen in comparison to females from fields under organic and integrated management. Wider posterior part of the carapace and less protruded frontal part was detected in female spiders from fields under integrated and conventional management. These results can be explained by faster growth as a consequence of pyrethroid hormetic effects on female individuals. In male spiders significant morphological differences between individuals collected from the different experimental fields were not observed, probably due to their higher mobility.

**Key words:** agriculture, pyrethroids, oilseed rape, spiders, *Oedothorax apicatus*, morphology

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## **Insecticide application in oilseed rape with dropleg technique – Impact on insect pests and their parasitoids in oilseed rape**

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**Abstract:** The use of the innovative dropleg technique for insecticide application at full flowering in oilseed rape is progressively postulated by bee keepers in Germany and is a tool for a safer pesticide application. The advantageous feature of dropleg technique is that the spray nozzles hang below the flowering canopy. Thus, the flowering parts of the plant are unaffected by active ingredient thereby reducing harmful effects of pesticides on beneficial insects such as honey bees and wild pollinators. Residues of plant protection products in honey and bee products can be decreased significantly and efficacy of dropleg technique against the fungal disease *Sclerotinia sclerotiorum* has been shown to be comparable to conventional application above the flowers (Weimar-Bosse *et al.*, 2017).

At the JKI field trials were done from 2016-2018 with the aim to investigate the effects of different insecticides on important insect pests during the flowering stage of oilseed rape and to compare the efficacy of dropleg with conventional spraying technique. Examined insect pests were: pollen beetle (*Brassicogethes aeneus*), seedpod weevil (*Ceutorhynchus obstrictus*) and brassica pod midge (*Dasineura brassicae*).

Field trials were conducted in a randomized block design with plots of at least 12 × 20 m with four replications in Wendhausen, nearby Braunschweig, Germany. The insecticides Biscaya (72 g thiacloprid/ha) and Mospilan SG (40 g acetamiprid/ha) were applied both with the traditional and the new dropleg technique at flowering stage (BBCH 65-67). In 2018 a new formulation of Mospilan was used including 42 g acetamiprid/ha. Six water trays (0.06 m<sup>2</sup>) were placed on the ground of each plot to measure both dropping of adult insect pests and migrating larvae. Trays were emptied once a week until harvest and insect pests were identified and counted in the laboratory. On two occasions (BBCH 75/BBCH 80-85) 12 plants per plot were randomly picked and pod assessment was carried out to determine pod infestation with brassica pod midge and cabbage seed weevil. Furthermore, two photoelectors (each 0.25 m<sup>2</sup>) were placed in each plot to calculate the emergence of new generation insect pests (Heimbach *et al.*, 2016).

An additional focus examined the effects of both techniques on the abundance of parasitoids within the oilseed rape crop. In 2017, insects were collected with a vortis section sampler (a modified backpack blower (STIHL)) several times from the day of application until 9 days after application to compare the number of parasitoids present in the crop as a measure of the effect of the different treatments/application methods. Moreover, larvae of pollen beetle were dissected to determine the parasitism rate according to Brandes *et al.* (2017).

Brassica pod midge occurred in the trial plots in all years whereas seedpod weevil was only present in a higher abundance in 2018. Conventional application of Biscaya significantly reduced the proportion of pods infested by first generation of *D. brassicae* in all years.

Mospilan showed similar efficacy only with the new formulation in 2018. Application of Biscaya with dropleg technique had weak effects on the number of infested pods, however, numbers of larvae dropping to the ground was reduced by about the same level with both application techniques in 2016 and 2017.

Parasitism rates by the hymenopteran parasitoid *Tersilochus heterocerus* of pollen beetle larvae were between 50 and 90 percent in control plots in all years. A positive effect of dropleg technique on *T. heterocerus* was recognized with a higher parasitism rate of pollen beetle larvae observed in 2017. Three days after application, plots treated with dropleg technique showed a similar parasitism rate to the control plots while the parasitism rate in conventionally treated plots was reduced.

Summarizing, efficacy of insecticides on insect pests is reduced with the novel dropleg technique compared to conventional application. At higher pest pressure conditions control of seedpod weevil and brassica pod midge with dropleg technique may not be sufficient.

**Key words:** dropleg technique, oilseed rape, pests, parasitoids

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**Pathology Session 1 A:  
Clubroot transcriptomes  
and host resistance**





## Overview of the clubroot incidence and variation in the pathotypes of *Plasmodiophora brassicae* populations in Europe

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**Abstract:** *Plasmodiophora brassicae* has recently become one of the most damaging pathogens to oilseed rape (OSR) cultivation in Europe. Questionnaires submitted by farmers and extension services revealed short rotation (once in 2-3 years) of the crop in 70% of fields. Frequency of OSR in the rotation significantly correlated with the incidence and prevalence of clubroot disease. Although there was a significant negative correlation between the disease index and soil pH, the occurrence of clubroot was not restricted to fields with highly acidic soils. Characterization of *P. brassicae* populations on the European Clubroot Differentials (ECD), and classification by the differential hosts of Williams or Somé *et al.* revealed that pathotypes: ECD 16/31/31 and 16/14/31; 4, 6 and 7; and P1 and P3, respectively, are predominant in central Europe. Several populations were found that could overcome the resistance of cv. Mendel, the first cultivar of OSR bred for resistance to clubroot.

**Key words:** clubroot, *Brassica napus*, disease incidence, physiological race

### Introduction

*Plasmodiophora brassicae*, a member of the Rhizaria kingdom, is one of the most damaging pathogens of OSR. In the last few years it has become increasingly economically important worldwide, including in different countries in Europe. Clubroot disease has been monitored by collaborators through field surveys. This study aimed at the recognition of the current incidence of the disease, its possible causes and the variation and distribution of the pathotypes of *P. brassicae* populations in the regions of the most intensive cultivation of OSR.

### Material and methods

The study was made in 2017 in seven European countries, including the Czech Republic, Denmark, France, Germany, Poland, Sweden and the United Kingdom, both in England and

in Scotland. Infected plants and soil samples were collected randomly from clubroot-infested fields in different countries. Location, soil type, plant genotype and rotation regime were recorded for each field. The pH-value of each soil sample was measured using routine procedures. The presence of *P. brassicae*-resting spores inside the soil was assessed by a standard greenhouse bioassay.

Pathotype classification of the *P. brassicae*-populations was conducted on two or three differential sets: the classification system of Williams (1966), the European Clubroot Differential set developed by Buczacki *et al.* (1975) and the set of Somé *et al.* (1996) in Czech Republic, France, Germany, Poland and the United Kingdom. Additionally, the degree of virulence of the collected isolates was analysed on the clubroot-resistant OSR cv. Mendel.

## Results and discussion

The symptoms of clubroot were widely spread and they occurred on OSR in different regions of all countries in the study. The studies revealed, besides favourable weather conditions for plant infection, clubroot was a major issue when OSR was grown in short rotations (once in 2-3 years). This could lead to a rapid increase in clubroot incidence and severity. A significant negative correlation was found between soil pH and the disease incidence in infested fields.

The pathotype distribution varied between the countries. In the Czech Republic and Poland, there were nine pathotypes according to the evaluation system by Williams, four pathotypes based on the differentials of Somé and 15 with the ECD set. In Germany, five pathotypes were found according to the evaluation system of Somé and 20 virulence patterns according to the ECD set. In France, six different pathotypes were classified according to the set of Somé. In the UK, there were 11 virulence groups according to the ECD set. According to the differential set elaborated by Williams (1966) the most common pathotypes in central Europe were 4, 6 and 7. According to the system developed by Somé *et al.* (1996) pathotypes P1 and P3 were the most frequent. Based on studies using the differential set elaborated by Buczacki *et al.* (1975) the most frequent pathotypes in *P. brassicae* populations were ECD 16/31/31 and 16/14/31.

While most of the *B. rapa* genotypes from the ECD set were completely resistant against Polish, Czech and many German *P. brassicae* populations, several populations were found in the UK and Sweden that could overcome the resistance of ECD 01, ECD 02 and ECD 03. In Sweden some populations were identified that could break down the resistance in all ECD *B. rapa* hosts. In some regions of Denmark, France, Germany and Poland a high number of isolates belonged to P1(+) were found which could overcome of the resistance gene(s) present in cv. Mendel.

The main control method used in all countries is the growth of cultivars resistant to clubroot. Some isolates could break the resistance of cv. Mendel, the first cultivar of OSR resistant to *P. brassicae*. These virulent populations were present in different geographical areas, which poses a new threat to OSR cultivation in Europe.

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## Clubroot disease and its biocontrol using host resistance

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**Abstract:** Clubroot disease will be introduced and its major control means will be described. Host resistance has a central role for the mitigation of clubroot and relevant examples will be described. Due to the strong race-differentiation of the pathogen clubroot resistance requires constant monitoring for new pathotypes and the acquisition of additional resistance sources with broad-spectrum efficacy.

**Key words:** Clubroot, integrated control, resistance

### Introduction

Clubroot disease is a major threat to all plants belonging to the Brassicaceae. It is caused by the strictly biotrophic protist *Plasmodiophora brassicae*, that is infecting host roots and grows as a plasmodium inside the host cytoplasm. Prior to sporulation, the typical symptoms develop, which are swollen, tumorous roots that have lost their concentric organization and impair the transport of water and nutrients. Upon decay, every infected root releases billions of highly persistent resting spores that pose a long-lasting risk for subsequent crucifer crops. Heavy clubroot infections can lead to complete losses of crops such as oilseed rape or crucifer vegetables. Due to the strong increase in oilseed rape or canola cultivation, clubroot has developed into a high priority disease in many cropping regions. Facing the risk that is caused by increasing inoculum loads, growers have to make detrimental agronomic decisions on crop rotations that affect not only the major crops but also break crops such as mustard and oil radish. At present, clubroot is controlled most effectively by the use of clubroot resistant (CR) cultivars. CR accessions can be found in major and minor crops. Most CR sources are race-specific, and European field isolates from *P. brassicae* display great variation. At present, CR genes from stubble turnips (*B. rapa*) are most effective and widely used in resistance breeding of different *Brassica* crops, such as Chinese cabbage, oilseed rape, and *B. oleracea*. A single, dominant CR gene having a race-specific effect was transferred to the *B. napus* cultivar ‘Mendel’ and is now widely used in cultivars derived from it (Diederichsen *et al.*, 2014; Fredua-Agyeman *et al.*, 2018). Compatible *P. brassicae* populations for the ‘Mendel’ resistance were present in some European areas already before its release and have spread slowly with the use of resistant cultivars. Therefore, CR will need improvements and additional means to support sustainable control. New CR sources need to be identified and combined and introgressed into the respective crop.

## Material and methods

Gene bank accessions and breeding material of *Raphanus sativus* were screened using field isolates originating from *R. sativus* or *B. napus* using a standard infection protocol (Diederichsen and Sacristan, 1996). Intergeneric hybridizations between *B. napus* and *R. sativus* were supported by ovary and ovule culture using culture conditions as described in Diederichsen and Sacristan (1994). Hybrid character was confirmed using SSR markers according to Lowe *et al.* (2004).

## Results and discussion

Most tested radish cultivars did show CR against *P. brassicae* isolates originating from *Brassica* that makes this species a valuable resistance source for *Brassica* crops. Screenings with an isolate showing strong virulence on radish cultivars revealed additional CR sources in *Raphanus* that can be used to generate highly resistant oil radish break crops.

Intergeneric hybrids were generated between *B. napus* and radish. Clonal plants of the hybrids were tested in three tests with two *P. brassicae* isolates (Table 1).

Table 1. Tests of clonal plants of the hybrids with two *P. brassicae* isolates.

		<i>P. brassicae</i> Isolate		
<b>Host</b>		ÜR14	ÜR14	PbRaph2
Mean score (0-3)	Radish	3	2.6	0
	Oilseed rape	0	2.6	3
	Yellow seeded Sarson (susc. control)	3	3	3
Number of	Resistant hybrids (score < 0.5)	33	43	28
	Hybrids with medium reaction (0.6-1.5)	25	7	27
	Susceptible hybrids (1.6-3.0)	40	41	53

Using embryo rescue 112 intergeneric hybrids were generated and confirmed as true hybrids and most of these have been tested for CR. The two isolates (ÜR14 and PbRaph2) show differential reactions on radish and *B. napus*, with ÜR14 being one of the very seldom isolates that display specific virulence on radish. The hybrids were segregating for CR, the two tests with isolate ÜR14 correlated stronger ( $R_s = 0.78^{**}$ ) with each other than with isolate PbRaph2. An isolate showing virulence on CR hosts from both species is unknown and it remains to be shown whether it is possible for *P. brassicae* to combine these virulence features. Resistant hybrids have been selected for further introgression. The different CR sources in *Raphanus* help to achieve broad-spectrum and highly efficient CR. They can be used to control clubroot infections by pyramiding CR in *Brassica* and radish crops, the latter being a candidate to develop a catch crop that can reduce inoculum levels in infested soils.

## Acknowledgements

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## Analyses of clubroot transcriptomes

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### Highlights

- Plant defence genes are down-regulated in clubroots.
- Symptomless roots of clubroot infected plants show an increased defence response.
- Effector gene candidates might be responsible for those differences and can be investigated using FISH.

### Introduction

The obligate biotrophic protist *Plasmodiophora brassicae* causes clubroot disease, one of the economically most important diseases of *Brassica* crops. Despite its importance, relatively little is known about infection strategies and host defence mechanisms compared to fungal and bacterial plant pathogens (Schwelm *et al.*, 2018). The aim of this study was to elucidate some of the interactions between *P. brassicae* and its host. We analysed the transcriptomic changes of different root tissue of infected plants from field samples. A FISH method was used to visualize pathogen effector candidates' expression in host cells to get an indication of their potential role in the disease process. We confirmed that massive changes transcriptomic changes in infected plant tissue occur and show the potential of combining *in situ* gene expression studies and transcriptomics to understand the interplay between *P. brassicae* and hosts.

### Material and methods

*Brassica oleracea* var. *gongylodes* roots were collected from plant of a heavily *P. brassicae* infested farm plot. The material was washed and stored in RNA later prior RNA extraction using the Qiagen RNeasy Plant Mini Kit. Poly(A) selected RNA was sequenced on an Illumina HiSeq 2500 platform in 125 bp paired-end read mode at VBCF Vienna. All reads were quality checked and trimmed. Reads of at least 75 bp length were kept for further analyses. High quality reads were assembled *de novo* using Trinity and expression estimation was performed using RSEM. Predicted genes were annotated using TransDecoder (<https://github.com/TransDecoder>). Annotation was performed using InterProScan ([www.ebi.ac.uk/interpro/](http://www.ebi.ac.uk/interpro/)), Mercator for MapMan analysis (<http://mapman.gabipd.org>), KAAS to obtain KEGG Orthology ([www.kegg.jp](http://www.kegg.jp)), and blasting sequences against NCBI nr database. Assembled transcripts were processed with edgeR to obtain log<sub>2</sub>-fold changes between disease stages. Pathogen and host transcripts were separated by matching them to either CDS of *B. oleracea* (Liu *et al.*, 2014) and other available *Brassica* species and available

*P. brassicae* CDS data (Schwelm *et al.*, 2015, Rolfe *et al.*, 2016) using a custom made database.

Thin sections of clubroots from *Brassica* host grown in pots were analysed using a FISH (Fluorescence *in situ* hybridisation) method based on (Duncan *et al.*, 2016). This method was used for specific gene expression for a SA-methylating *P. brassicae* PbBSMT gene (Ludwig-Müller *et al.*, 2014) and another potential *P. brassicae* effector. Microscopical analyses were performed using a Leica LSM SP5 confocal laser-scanning-microscope.

## Results and discussion

In 2016, outbreaks of clubroot disease were seen at many sites in Tyrol, with no history of *Brassica* crops in the last 10 years or longer. High precipitation and low soil pH values of pH 5.60 (despite liming) provided good conditions for clubroot development on a large variety of *Brassica* vegetable crops. We used those field samples to investigate the interplay between *Brassica oleracea* var. *gongylodes* and *P. brassicae* in a real agricultural scenario.

We analysed differences in the transcriptome of clubroots and symptomless roots of the same plant. The RNAseq profiles did differ in the expression of genes related to cell wall stability. Similarities between the RNAseq pattern of symptomless tissue and resistant plants were identified. Roots without clubroot symptoms showed generally a higher expression of defence related genes. This implied that the defence of the plant is down-regulated by *P. brassicae* in infected gall tissue. How this is achieved remains unclear, but *P. brassicae* is known for re-programming host metabolism (Ludwig-Müller *et al.*, 2009).

The PbBSMT gene encodes for a secreted protein, which's likely function is to reduce the SA response in infected tissue (Ludwig-Müller *et al.*, 2015). Induced PbBSMT expression was detected during the sporulation stage of *P. brassicae* using FISH. Another highly expressed secreted protein was mainly expressed in plasmodia in earlier studies (Schwelm *et al.*, 2015), which was confirmed by FISH. However, no function for this effector candidate can be predicted, as no known functional domains are present. The lack of known functional domains in many predicted genes of *P. brassicae* (Rolfe *et al.*, 2016, Schwelm *et al.*, 2015) complicates the interpretation of their function. In addition, there is no transformation system for *P. brassicae* available yet, to do gene replacement studies. But transcriptomic studies combined with tissue and cell specific microscopical methods – as FISH presented here – will facilitate the interpretation of the role of different genes in this pathosystem. Further studies could show if in different *Brassica* hosts similar genes are similar regulated when infected by a *P. brassicae* and might reveal host specific defence responses.

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## Molecular analysis of bulk soil samples for *Plasmodiophora brassicae* inoculum and spatial distribution of resting spores in a patchy infested winter oilseed rape field

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**Abstract:** Molecular analysis of pathogen inoculum in soils is often limited by inhibitors present in different soil types which are not efficiently extracted by the applied DNA extraction method. This may lead to inefficient or no amplification of target sequences. A further drawback of many methods is the limitation of sample sizes (mg scale) which can be processed. Accordingly, obtained results may be biased and repetitions possess very high standard deviations. A very heterogeneous distribution of the target organism in the soil may be a main reason for this. Scaling up sample sizes is a promising way to reduce or even eliminate this bias. Altogether, the use of up-scaled sample sizes in combination with extraction methods that can cope with PCR inhibitors may overcome the described problems and may lead to a much more precise molecular determination of soil inoculum. Following this idea, we tried a large scale and silica based method described by Woodhall and co-workers (2012) using soil sample sizes of 250 g for the detection of the club root pathogen *Plasmodiophora brassicae*. The extraction is based on the use of a commercial paint mixer in which homogenization of the samples is achieved. Different published diagnostic primer sets will be tested and assessed for both specificity and sensitivity. The best set will be used for further studies. In following model experiments the method will be validated on six different soil-types. Soil samples will be artificially contaminated with different concentrations of *P. brassicae* resting spores. The assay will finally be applied to analyze a field site managed by our University close to Göttingen (Rodetal) showing a patchy infestation with the soil borne, obligate protist *P. brassicae*. The patches were measured by GIS techniques for follow-up studies. The spatial distribution of inoculum will be investigated in connection to disease formation on the spot. For this purpose, we took soil samples directly in the affected oilseed rape field in spring 2017. Samples were taken in clubroot patches and in different distances to the borders of these patches. We expect to see gradual changes of soil inoculum depending on the sampling site. In parallel, pH values of the individual samples will be measured which may help to explain the patchiness of the disease in this field. Finally, pathotype analysis on both, the ECD differential set and the differential set described by Somé *et al.* (1996) will be performed in order to describe the local races present at this site. Available results of this project will be presented at the meeting.

**Key words:** *Plasmodiophora brassicae*, detection, PCR, bulk soil samples

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## Identification of clubroot resistance sources from world gene bank accessions

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**Abstract:** This study describes the resistance of 300 accessions of various species of *Brassica* to clubroot disease caused by *Plasmodiophora brassicae*. The examined lines originated from the Center for Genetic Resources for Food and Agriculture in the Netherlands (CGN) and Plant Gene Resources in Canada (CN). Studied plant materials included *Brassica oleracea*, *B. rapa*, *B. napus* and *B. villosa*. Six pathogen pathotypes, collected in Poland were used for the plant inoculations – P1, P1(+), P2, P3, P4 and P5. The experiments were carried in greenhouse conditions. In most cases the observed resistance was pathotype specific and only one *Brassica* form, three accessions of *B. oleracea*, were resistant to all the examined pathotypes of *P. brassicae*, including the P1B pathotype, which is the most damaging to *Brassica* plants in Poland. Resistance to clubroot was rare and mostly pathotype-specific.

**Key words:** clubroot, *Plasmodiophora brassicae*, resistance, gene bank

### Introduction

Clubroot, caused by the protozoa pathogen *Plasmodiophora brassicae* Woronin, is a damaging disease of *Brassica* crops (Dixon, 2009) and a threat to oilseed rape production both in Poland and worldwide (Jedryczka *et al.*, 2013). For the last few years the pathogen has posed a serious challenge to growers of oilseed rape in Poland. According to recent reports the pathogen is present in *ca.* 250 000 ha under cultivation of oilseed rape and its further expansion is still being observed (Konieczny, 2012).

The pathogen is very persistent in soils and can retain infectivity even after several years in the soil. The rate of spread of the disease may be extremely high. It was found that within three years from the onset of disease symptoms on a small number of plants, in favorable conditions, the pathogen can take over the whole field. This situation appears when no crop rotation is used, which may happen in regions with intensive cultivation of oilseed rape (Rimmer *et al.*, 2007). Agrotechnical methods, such as liming, are able to decrease the levels of plant infection, but they are unable to stop the disease. Reducing the frequency of *Brassica* crops in a rotation significantly delays the initial incidence of clubroot, although this is not a preferred option for growers faced with an increasing demand for plant oils. In addition, crop rotation alone will not eliminate the disease at infested sites in the short term. Cultivar resistance is a cornerstone for the management of clubroot disease (Diederichsen *et al.*, 2009).

Except for *B. juncea* and *B. carinata*, resistant accessions can be found in all major *Brassica* crops (Diederichsen *et al.*, 2009). Resistant Chinese cabbage (*B. rapa* subsp. *pekinensis*) and oilseed rape (*B. napus*) have been developed in Japan (Kamei *et al.*, 2010) and Europe (Diederichsen *et al.*, 2006). Almost all clubroot-resistant (CR) *Brassica* crops

have single gene-based resistance that is often race- or pathotype-specific although some race-independent resistant accessions have been found in *B. oleracea* (Diederichsen *et al.*, 2009). Most of the highly resistant forms were found in *B. rapa*, predominantly among turnip varieties (Peng *et al.*, 2015). Oriental crucifer vegetables like Pak Choi and Chinese cabbage also showed strong resistance, but the origin of clubroot resistance in these accessions is not known (Peng *et al.*, 2015). Clubroot resistance was also identified among accessions of *B. nigra* and *B. oleracea* (Hasan *et al.*, 2012).

The aim of this work was to identify sources of genetic resistance to clubroot in *Brassica* accessions deposited in world gene bank collections. The accessions evaluated in this experiment originated from the Centre for Genetic Resources in the Netherlands (CGN) and Plant Gene Resources in Canada (CN).

## Material and methods

The collection of 300 *Brassica* lines included *B. oleracea* (223), *B. villosa* (1), *B. napus* (34) and *B. rapa* (42) and standards for resistance (*B. napus* cv. Mendel and Tosca) and susceptibility (*B. napus* cv. Californium).

The bioassay screening for susceptibility/resistance to clubroot was performed as follows. The seeds of tested genotypes, 30 plants for each interaction (plant genotype × pathogen pathotype), were sown to soil with pH 5.8 and grown at 20-22 °C day/16-18 °C night with a 12 h photoperiod for five days. After this time the seedlings, which were then at the early stage of cotyledon development were inoculated with the spores of *P. brassicae* (3 ml of  $1 \times 10^7$  spores/ml per plant) obtained from clubs, originating from previously propagated plant materials. The inoculum was applied to the soil with syringe, close to the roots of inoculated plant. The artificial infection was done with the pathotypes P1, P2, P3, P4, P5, designated according to Somé *et al.* (1996) and P1(+) pathotype virulent on cv. Mendel.

The evaluation of resistance was done 6 weeks after planting, using a 0-4 scale, where 0 was no disease symptoms, 1 was a root shorter than the control one, distorted or curly, 2 was a root with very small clubs, mostly not bigger than 2-4 mm diameter, 3 was a root system partially transformed to a club and 4 was a root system completely transformed to a large club. The accessions were regarded as highly resistant when no clubs formed on the main root or on multiple lateral roots. They were evaluated as partially resistant when their average score was equal '2', which means that some small clubs formed on the root system.

## Results and discussion

Out of 300 accessions tested there were seven accessions with resistance to all tested pathotypes of *P. brassicae*, but only the accessions CGN11150, CGB14078 and CGN15227 of *Brassica oleracea* showed high resistance to all pathotypes of clubroot. The accessions CGN06903, CGN11130 and CGN14048 were also resistant to the pathogen, with high or partial resistance, depending on the pathotype. The accession CGN11130 showed partial resistance to all tested pathotypes. Only one accession, CGN107759, was resistant to five pathotypes, including the pathotype P1B. Five accessions showed high or partial resistance to four pathotypes and their composition was different each time. Two accessions were resistant to three pathotypes and again in each case this resistance was to different composition of pathotypes. Three and five accessions respectively were highly/partially resistant to one or two pathotypes. The cultivars Mendel and Tosca were resistant to all pathotypes except P1(+).

The cultivar Californium was susceptible to all tested pathotypes. The results obtained in this study concerning the resistance of tested accessions obtained from CGN and CN collections are presented in Table 1.

Table 1. List of *Brassica* accessions originating from the Centre for Genetic Resources (CGN) in the Netherlands and the Plant Gene Resources (CN) in Canada, resistant to different pathotypes of clubroot.

Species	Accession	P1	P1(+)	P2	P3	P4	P5
<i>Brassica oleracea</i>	CGN06903	XX	X	X	XX	X	XX
	CGN18947	XX		X	X		XX
	CGN11062			XX		XX	
	CGN11127	XX					
	CGN11128	XX					
	CGN11129			XX			X
	CGN11130	X	X	X	X	X	X
	CGN11131	XX		XX		XX	XX
	CGN11138			X			
	CGN11150	XX	XX	XX	XX	XX	XX
	CGN14048	XX	X	XX	XX	XX	XX
	CGN14078	XX	XX	XX	XX	XX	XX
CGN15227	XX	XX	XX	XX	XX	XX	
<i>B. napus</i>	CN114234	XX		XX	XX		XX
<i>B. rapa</i>	CN105404				XX		XX
	CN105405			XX	XX	XX	XX
	CN105406	X		XX	XX	XX	
	CN107727			XX	XX		XX
	CN107744			XX		XX	XX
	CN107759	XX		XX	XX	XX	XX
	CN107764	XX				XX	

XX- full resistance, X- partial resistance

Crisp *et al.* (1989) evaluated approximately 1000 *B. oleracea* accessions and confirmed the existence of clubroot resistance in several European kales and cabbages, with a new source of clubroot resistance identified in the cabbage 'Eire'. Of the 48 *B. oleracea* accessions tested by Hasan *et al.* (2012), five showed resistance to pathotype 3 and three showed resistance to pathotype 5. In the study of Peng *et al.* (2015) five of the 30 *B. oleracea* accessions tested were resistant to pathotype 3, with the cabbages 'Kilaherb' and 'Tekila' being highly resistant to all five pathotypes found in Canada.

Among *B. rapa* all tested accessions were partially resistant. Hasan *et al.* (2012) reported that all five of the turnip accessions they tested were highly resistant to the five *P. brassicae* pathotypes found in Canada and it appears that turnips carry useful clubroot resistance genes.

In this experiment the majority of *Brassica* accessions tested were highly susceptible to *P. brassicae*. Only 7% of lines exhibited any resistance and just 2% were resistant to all pathotypes. The resistance found in CGN11150, CGN14078 and CGN15227 was strong and broad-spectrum providing protection against all tested pathotypes, including P1(+) – the most aggressive pathotype occurring in Poland.

## Acknowledgements

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## Introduction of clubroot resistance to rapeseed through interspecific hybridization

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**Abstract:** The aim of this study was to identify the sources of genetic resistance to clubroot and to obtain the interspecific hybrids with increased level of resistance. The plant material consisted of five high yielding cultivars (Anderson, Arsenal, Andromeda, Californium and Monolit) of *Brassica napus* and selected *Brassica rapa* genotypes with increased resistance to *Plasmodiophora brassicae*. All interspecific hybridizations were performed with the application of an *in vitro* embryo culture. The effectiveness of interspecific crosses varied widely depending on the genotype used as a pollinator. The lowest efficiency was observed in crosses between *B. napus* cv. Arsenal and *B. rapa* var. *rapa*.

**Key words:** clubroot, *Plasmodiophora brassicae*, interspecific hybridization, *Brassica napus*, *Brassica rapa*, *in vitro* embryo culture

### Introduction

Amphidiploid rapeseed as a very important oil plant became a widely cultivated crop in many countries worldwide. Obtaining forms with improved traits is highly desirable and one valuable tool in this search is interspecific hybridization which enables the transfer of useful traits such as seed quality and resistance to diseases. An increasingly important disease for brassica production is clubroot, caused by the soil-inhabiting protist *Plasmodiophora brassicae*, which is highly damaging to oilseed rape and vegetable brassicas (Niemann *et al.*, 2015). The main sources of clubroot resistance used up to date originate from different species of the genus *Brassica*, including *B. rapa* (A-genome), *B. oleracea* (C-genome) and *B. napus* (AC-genome). The aim of this study was to characterize the resistance of parental *Brassica* genotypes to clubroot and to obtain interspecific hybrids with increased levels of resistance.

### Material and methods

Experimental material consisted of five *B. napus* cultivars and selected *B. rapa* genotypes with increased resistant to clubroot (Table 1). The study was conducted using a bioassay with *P. brassicae* isolates belonging to the pathotypes P1-P5. Parental genotypes chosen for the crossing programme showed increased resistance to the most damaging pathotype P1. All hybridizations were performed with the application of an *in vitro* embryo culture according to the method described by Wojciechowski (1998). The immature embryos were isolated from young siliques at different developmental stages, i. e. heart and early and late torpedo, 14-19 days after pollination. Under the stereoscopic microscope the ovules were removed



aseptically by cutting them lengthwise along the suture. The isolated embryos were transferred to White (W) or Murashige & Skoog media and incubated at  $26\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$  at 16 h light phase and 8 h dark phase. According to this protocol for regeneration, after 3 weeks the embryos were transferred onto fresh MS or MS medium modified by Keller (MSk). When the embryos had grown into plantable seedlings, they were transferred for rooting on Nitsh & Nitsh ( $\text{H}_3$ ) medium. Rooted seedlings were transplanted directly to the soil and after 10 weeks of vernalization grown further in the glasshouse.

## Results

The effectiveness of interspecific crosses varied widely depending on which species were used as a pollinator (Table 1). The lowest efficiency was observed in crosses between *B. napus* cv. Arsenal and *B. rapa* var. *rapa*. In this case, there were no seeds set on the plant but in *in vitro* embryo culture the efficiency measured by the number of regenerated hybrid plants ranged from 0% to 60%.

Table 1. The effectiveness of interspecific crosses between *B. napus* var. *oleifera* ( $2n = \text{AACC} = 38$ ) and *B. rapa* ( $2n = \text{AA} = 20$ ) expressed by the number of obtained embryos and the number of regenerated plants (%).

Cross combination		Number of pollinated flowers	Number of siliques	Number of isolated embryos	Ovules with embryos (%)	Number of embryos per silique	Number of regenerated plants	Effectiveness of <i>in vitro</i> cultures (%)
♀	♂							
<i>B. napus</i>	<i>B. rapa</i>							
Anderson	<i>B. rapa</i> ssp. <i>pekinensis</i>	25	14	16	84.2	1.1	6	37.5
Arsenal		20	5	46	95.8	9.2	10	21.7
Andromeda		27	15	47	64.4	3.1	12	25.5
Californium		63	33	56	65.9	1.7	18	32.1
Monolit		21	11	29	82.8	2.6	15	51.7
Anderson	<i>B. rapa</i> var. <i>rapa</i>	17	9	10	83.3	1.1	6	60.0
Arsenal		43	0	0	0	0	0	0
Andromeda		32	6	0	0	0	0	0
Californium		12	2	2	22.2	1	0	0
Monolit		18	16	7	77.8	0.4	0	0
<b>Total</b>		<b>278</b>	<b>111</b>	<b>213</b>	<b>73.</b>	<b>1.9</b>	<b>67</b>	<b>31.4</b>

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**Pathology Session 1 B:  
IPM strategies for clubroot control**



## **Integrated management of clubroot in WOSR using resistant cultivars in soils with different inoculum levels**

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**Abstract:** Clubroot disease is a serious threat to OSR production in Sweden and genetic resistance is the most important factor in a cropping strategy. The objective of a new project started in 2017 is to provide an improved decision support and guidelines for growing clubroot resistant (CR) cultivars in infested soil.

**Key words:** *Plasmodiophora brassicae*, qPCR, soil samples, resistant cultivars

### **Introduction**

Clubroot, caused by the soil borne pathogen *Plasmodiophora brassicae*, is a serious threat to oilseed rape (OSR) production. The increasing proportion of clubroot infected arable land in Sweden has serious consequences for OSR growers. Recently compiled analyses from farm soil samples showed occurrence of clubroot in 49% of 201 farm soils analysed (Kihlstrand, 2016).

Clubroot is recognized as the most serious soil-borne disease of *Brassica* oilseed and vegetable crops world-wide associated with appreciable yield losses (Dixon, 2009). Control of clubroot is particularly difficult due to the persistence of the pathogen in soil (Wallenhammar, 1996) and makes disease control by means of crop rotation difficult. Breeding brassica crops for resistance has been the ultimate objective and the first clubroot resistant (CR) variety, with a low yield penalty was released 2001 in Germany. The resistance is based on the dominant gene effective against most field populations of *P. brassicae* (Diederichsen *et al.*, 2014).

A new project aiming at developing a concept for integrated production of winter OSR supported by DNA technology started in 2017. The objective is to provide an improved decision support and guidelines for growing winter OSR in fields where *P. brassicae* DNA occurs.

### **Material and methods**

#### ***Test of agronomic performance of resistant varieties in field soils with different inoculum levels***

Field trials were performed in a randomized block design with four replicates in four fields at different levels of contamination of *P. brassicae*. The field experimental sites were selected in south (55°N, 14°E) and in south central (59°N, 15°E) Sweden. Three resistant varieties of WOR were selected and seeded together with a mixture of susceptible varieties at the earliest

on 9 August and the latest on 25 August 2017, due to high precipitation. Soil temperature is monitored continuously in each trial with data logger equipment.

#### ***Disease assessment***

Disease incidence and disease severity were assessed plot-wise in late November and a disease severity index was calculated.

#### ***Soil sampling and mixing***

Ten soil samples were randomly collected plot wise prior to seeding the experiment with a soil auger to 15 cm depth. The soil samples were mixed according to previously developed routines, and a sample of 50 g was withdrawn for DNA-analysis.

#### ***Analyses***

Quantification of *P. brassicae* by qPCR analysis was performed according to Wallenhammar *et al.* (2012).

#### ***Bio assays***

Bio assays were carried out in a greenhouse for a six week period to ensure optimal infection. The varieties performing in the field trials were tested.

## **Results and discussion**

The fields selected for field experiments showed *P. brassicae* at levels ranging from 5000 to 2.5 million target copies per g soil at pre-sampling in July 2017. Soil samples collected plot-wise immediately to seeding the trials verified pre-sampling results particularly in fields with the highest and lowest inoculum levels. For the other fields a variation between plots was larger as the pathogen has a patchy prevalence. Plants sampled in late autumn show severe infections of susceptible cultivars whereas the CR-cultivars seem to resist severe (> 30% infected plants) infections in all of the fields. Preliminary results will be discussed as soil DNA-analyses will be correlated with disease severity and yield.

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## Biological control of clubroot (*Plasmodiophora brassicae*) by an endophytic fungus

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**Abstract:** The clubroot disease caused by *Plasmodiophora brassicae* infects economically important crop species such as canola and causes high yield losses. The disease is difficult to control by chemical and cultural means. In a previous study from our laboratory *Acremonium alternatum*, a soilborne endophytic fungus and known biological control agent in other countries, showed a promising antagonistic effect in clubroot infected plants. The means by which *Acremonium* controls pathogens is not known so far. Presumably the fungus induces resistance mechanisms in the host plant and thus delays the development of the pathogen in different host plants (Doan *et al.*, 2010; Jäschke *et al.*, 2010; Auer & Ludwig-Müller, 2014; 2015). We want to test this theory in the model plant *Arabidopsis thaliana* as well as the economically important crop species oilseed rape/canola (*Brassica napus*) and Chinese cabbage (*B. rapa*). To monitor the development of the infection within plants we use molecular methods as well as phytopathological techniques to address the questions how does *A. alternatum* induce tolerance / resistance in *Arabidopsis* to use this knowledge for other Brassica crops.

Pathogen material was the *Plasmodiophora brassicae* single spore isolate e3 (Klewer *et al.*, 2001), which was administered at  $10^7$  spores/ml. *Acremonium alternatum* was grown according to the protocol described in Jäschke *et al.* (2010), Doan *et al.* (2010) and Auer & Ludwig-Müller (2014) and the host plants were inoculated likewise. Host plants were *A. thaliana*, *B. rapa* ssp. *pekinensis* and *B. napus*. Plants were evaluated for disease symptoms 28 to 35 days, depending on the species, after inoculation using a disease rating with four classes according to Siemens *et al.* (2002) and a disease index was calculated. The endophyte was administered as live spores during different time points before and at the time point of inoculation with *P. brassicae*. Also an autoclaved spore suspension and a cell wall preparation (Vadassery *et al.*, 2009) was used. qPCR was carried out as described in Ludwig-Müller *et al.* (2017).

We have demonstrated that the fungus *A. alternatum* is present in *A. thaliana* as an endophyte, since we have detected its DNA in the upper plant parts after a root inoculation experiment (Jäschke *et al.*, 2010). In a first approach we could show that *A. alternatum* can slow down the development of the clubroot pathogen and we found lower disease indices after treatment with *A. alternatum*. For this, we have monitored gene expression of early and late expressed *P. brassicae* genes in different treatments (Jäschke *et al.*, 2010). We have tested different types of elicitors and have shown that *A. alternatum* is most effective when applied not at the same time but before inoculation with the clubroot pathogen *P. brassicae*. In addition, not only live spores, but also autoclaved spores of the fungus as well as a cell wall extract was able to reduce the clubroot symptoms. We have then investigated by qPCR the expression of different marker genes for either salicylic or jasmonic/ethylene pathways, and in addition also transcription factor and signaling genes. We could show that most likely the salicylic acid pathway is induced in the early time periods after co-inoculation with the two microbes, because of the upregulation of the *PR1* transcript. We are currently testing mutants

and overexpressors for some differentially expressed genes to verify the importance of those signaling pathways.

While the inducible genes during co-inoculation might be used to define targets for breeders, the long-term goal of our work is to develop an inoculum from *Acremonium* spores or spore parts which can be applied easily and constitutes an environmentally friendly and lasting method for the reduction of clubroot infections. Together with resistant cultivars additional biocontrol methods can increase the durability of resistance factors.

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## Seed coating with the fungal biocontrol agent *Clonostachys rosea* controls clubroot in oilseed rape

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**Abstract:** Clubroot, caused by *Plasmodiophora brassicae*, is a devastating disease in cruciferous crops and the problem is increasing, among others because the pathogen is very difficult to control. Therefore, new alternative control measures are needed and biological control could be such an option. *Clonostachys rosea*, isolate IK726, is a versatile fungal biocontrol agent and it was tested as seed coating against *P. brassicae* in two cultivars of oilseed rape. *C. rosea* IK726 reduced mean disease severity in both cultivars and furthermore also the frequency of plants with visible disease symptoms. Quantification of pathogen biomass by qPCR of *P. brassicae* DNA additionally showed that IK726 reduced this parameter and reductions were seen as early as 7 days after pathogen inoculation. Collectively, the results presented here show that *C. rosea* isolate IK726 can control clubroot under controlled conditions and that it could be considered a potential candidate for future field testing.

**Key words:** *Plasmodiophora brassicae*, *Brassica napus*, biological control, seed coating

### Introduction

Clubroot, caused by *Plasmodiophora brassicae*, is a well known pathogen of all cruciferous crops, with oilseed rape and vegetable Brassicas being the most important hosts. In recent years, the disease has emerged as a serious treat to these crops in Europe with up to 50% losses in, e. g., oilseed rape (Burnett *et al.*, 2013). This is mainly a result of intensification and changes in agricultural practices. The situation is further aggravated by the fact that the pathogen can survive for up to 20 years in the soil, making it virtually impossible to eliminate. Furthermore, even though resistance to the disease is known, currently only one gene is widely used and the pathogen populations have overcome this resistance in great parts of the intensive cultivation areas in Europe (Diederichsen *et al.*, 2014). The situation is exacerbated by the fact that the pathogen can survive in volunteer plants and cruciferous weeds and in addition, the extensive use of *Brassica* catch crops like oil radish may also have contributed to maintaining the inoculum potential of the pathogen. Therefore, management of clubroot require a consorted effort, addressing a range of control options. Host plant resistance will be of particular importance to control the disease, but alternative measures like biological control, using microorganisms, could also be a valuable tool in an IPM strategy.

Several biological control agents (BCAs) have shown potential for controlling clubroot as recently reviewed by Ludwig-Müller (2016). In most studies, bacterial or and fungal BCAs were applied by soil drenching or by mixing into the soil or growth medium in order to control the disease (Gossen *et al.*, 2016; Lahlali & Peng, 2014). However, biocontrol of

clubroot in field crops such as oilseed rape is probably only cost efficient if the BCA can be applied by seed coating. Previously, Peng *et al.* (2011) showed that seed coating with the commercial fungal product Prestop (*Clonostachys rosea*, syn. *Gliocladium catenulatum*) significantly reduced clubroot symptom severity at low inoculum pressure. However, the influence of host plant resistance in oilseed rape on symptom development has, to the best of our knowledge, not been studied for *C. rosea* or other fungal BCAs delivered by seed coating.

*Clonostachys rosea*, isolate IK726, isolated from barley roots (Knudsen, 1994), is a versatile biocontrol agent (Jensen *et al.*, 2007, Karlsson *et al.*, 2015, Jensen *et al.*, 2016), which can significantly control seed- and soil-borne diseases in field grown cereals and Chinese cabbage (Jensen *et al.*, 2001; Møller *et al.*, 2003).

The aim of this project was to investigate whether seed coating with IK726 could reduce development of clubroot in oilseed rape and in particular to study the effect of host plant genotype on the biocontrol efficacy. A series of plant bioassays with a susceptible and a resistant cultivar was conducted to determine effects of IK726 on early infection progress of *P. brassicae*, studied by the effect on symptom development as well as by quantification of pathogen biomass.

## Material and methods

### *Plants*

Two cultivars of winter oilseed rape were included in the experiments: cv. DK Exclaim and cv. DK Platinum from Dekalb, kindly provided by Monsanto Crop Sciences Denmark A/S. DK Platinum has resistance against *Plasmodiophora brassicae* whereas DK Exclaim has not.

### *Preparation of Plasmodiophora brassicae resting spore inoculum*

Roots of oilseed rape with severe clubroot symptoms were sampled from a commercial field near Køge, Denmark. Resting spores of *P. brassicae* were isolated from the clubs following the procedure of Sundelin *et al.* (2010) and spores stored at -18 °C until use.

### *Clonostachys rosea inoculum production and seed coating*

The biological control agent *C. rosea*, (isolate IK726) was cultured on potato dextrose agar. Spores were harvested in sterile distilled water from 2-week-old cultures and the concentration adjusted to  $1.5 \times 10^6$  spores/ml. Seeds were coated by soaking them in conidial suspensions ( $1.5 \times 10^6$  conidia/ml) of isolate IK726 (1:2 w/v) in 50 ml centrifuge tubes on a rotary shaker at 200 rpm for 10 min. The seeds were dried in laminar flow hood for 2 h before sowing.

### *Bioassays for disease reductions*

Growth medium for the plants consisted of a 3:1 (w/w) mixture of Pindstrup potting mix (Pindstrup Substrate no. 2, Pindstrup Mosebrug, Ryomgård, Denmark). A spore suspension of *P. brassicae* ( $10^4$  spores/ml) was mixed into 1.6 kg growth medium, resulting in  $6.5 \times 10^3$  resting spores of *P. brassicae* per g growth medium. Closed 1-l plastic pots (12 cm diameter, 13 cm high) were filled with 400 g growth medium. Sixteen seeds of either DK Exclaim or DK Platinum were sown in each pot. The seeds were either coated with *C. rosea* or treated with water (control). Four replications per treatment were arranged in a randomised block design. The pots were placed in a growth chamber under the following conditions: cycles of 16 h of light (Philips Master IL-D 36 w/865, France,  $200 \mu\text{E}/\text{m}^2\text{s}$ ) and 8 h of

darkness. Day and night temperatures were maintained at 20 °C (60% RH) and 15 °C (80% RH), respectively.

#### ***Assessment of clubroot symptoms***

Roots were harvested, rinsed in water and the crown roots examined for symptoms at 34 days after inoculation (dai), at the end of the experiments. Severity of the characteristic clubroot symptoms was scored, using the 0-5 severity scale of Wallenhammer *et al.* (2000): 0 = no galls; 1 = enlarged lateral roots; 2 = enlarged tap root; 3 = enlarged napiform tap root; 4 = enlarged napiform tap root, lateral roots healthy; 5 = enlarged napiform tap root, lateral roots infected. The average disease severity was calculated and results are presented in Figure 1. Disease incidence was also recorded and plants were either considered as healthy (score 0) or diseased (scores 1-5).

#### ***Quantification of *P. brassica* biomass in oilseed rape roots***

Samples for RT-qPCR were harvested at 7, 14, and 21 days after sowing. Roots were washed and subsequently lyophilized, ground in liquid nitrogen, using a mortar and pestle, followed by storage at -20 °C until use. DNA extraction took place as described by Sundelin *et al.* (2010), using the Qiagen DNeasy Plant Mini kit (Qiagen, Copenhagen Denmark). gDNA from purified resting spores was used as a reference for the qPCR.

DNA concentration was determined using a NanoDrop ND-1000 Spectrophotometer. Prior to performing the qPCR for quantifying the biomass, template DNA from each sample was diluted to a concentration of 10 ng/μl.

The qPCR assay was performed using the Agilent Technologies system (AriaMix Real-time PCR system) in 10 μl wells (in triplicate). One-μL template DNA was used and 5 μl SYBR Green-1 PCR master mix (Agilent Technologies) was used. The thermal cycling profile was one cycle of denaturation at 95 °C for 5 min, then 30 cycles each of 30 sec at 95 °C, 30 sec at 60 °C and 1 min at 72 °C, followed by one cycle of melting for 30 sec at 95 °C, 30 sec at 60 °C and 30 sec at 95 °C. The cycle threshold values ( $C_T$ -values) were determined by a serial dilution, ranging from  $10^7$  to  $10^0$  ng DNA of *Plasmodiophora brassicae*. The qPCR reactions were run using the amplification primers designed by Sundelin *et al.* (2010).

#### ***Statistical analysis***

Symptom scoring data as well as data on pathogen biomass represent continuous variables and these data were analysed by analysis of variance, assuming a normal distribution. Variances were stabilised by appropriate transformation of data if necessary. Data for disease incidence represent a discrete variable since it was recorded whether or not plants were diseased. Therefore, these data were analysed by Fisher's exact test. Hypotheses were rejected at  $P \leq 0.05$  and all data analysed using PC-SAS (release 9.4; SAS Institute, Cary, NC).

## **Results and discussion**

#### ***Disease severity***

Disease severity results are shown in Figure 1. The figure shows the mean of three independent experiments since all reacted similarly and there were no interactions between treatments and experiments. *Clonostachys rosea* application significantly reduced disease severity in both DK Exclaim and DK Platinum by 31% and 72%, respectively.

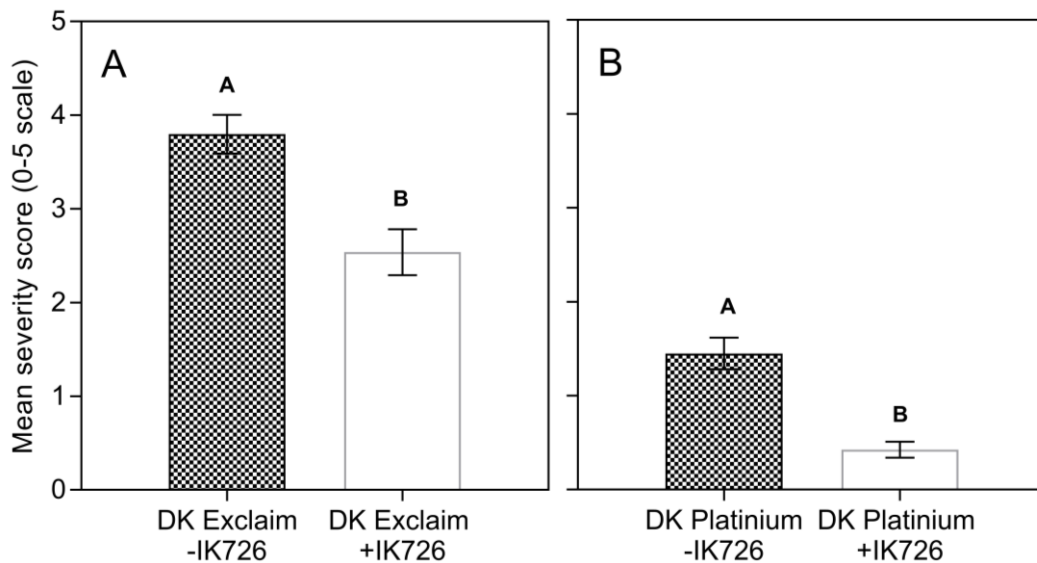


Figure 1. Clubroot severity assessment in oilseed rape using the scale of Wallenhammar *et al.* (2000) at 34 dai. A) DK Exclaim and B) DK Platinum. Seeds of each cultivar were either coated with *C. rosea* (marked IK726) or treated with water (control) and subsequently grown in medium inoculated with *P. brassicae*. Assessment was done visually at 34 dai. The values represent the means of three independent experiments (n = 80 plants). Means within each cultivar marked with different letters are significantly different. Error bars represent standard error of the mean.

### ***Disease incidence***

In addition to a reduction in disease severity after seed coating, *C. rosea* also reduced clubroot incidence (Table 1). Data are presented individually for three independent experiments since trials did not all react similarly.

For DK Exclaim (Table 1 A), all plants became infected by *P. brassicae* in all 3 experiments in the water-treated control. However, when seeds of this cultivar was coated with IK726, there was a significant reduction in number of infected plants in experiments 1 and 3, but not in experiment 2. For DK Platinum (Table 1 B), not all plants in the water-treated control was infected with *P. brassicae* (Table 1 B), but in all three experiments, coating with IK726 significantly reduced the percentage of infected plants. It is clear from the results that even at high pathogen inoculum level, coating with IK726 significantly reduced disease incidence.

### ***Quantification of P. brassicae biomass in oilseed rape roots***

Preliminary RT-qPCR data shows a significant reduction of biomass (pathogen DNA) of *P. brassicae* after coating with IK726 (Figure 2). In both cultivars, it was possible to detect a significant difference in biomass of pathogen DNA already at 7 dai when comparing between seed coating with IK726 compared to the control.

Table 1. Percent oilseed rape plants infected with *P. brassicae*. A) DK Exclaim and B) DK Platinum. Seeds of each cultivar were either coated with *C. rosea* (marked + IK726) or treated with water (-IK726) and subsequently grown in medium inoculated with *P. brassicae*. Assessment was done visually at 34 dai. The values were obtained from three independent experiments (n = 80 plants). Means within each cultivar marked with different letters are significantly different.

A) DK Exclaim	Exp. 1	Exp. 2	Exp. 3
-IK726	100.0a	100.0a	100.0a
+IK726	81.3b	93.3a	78.1b
B) DK Platinum			
-IK726	53.1a	68.8a	59.4a
+IK726	18.8b	18.8b	25.0b

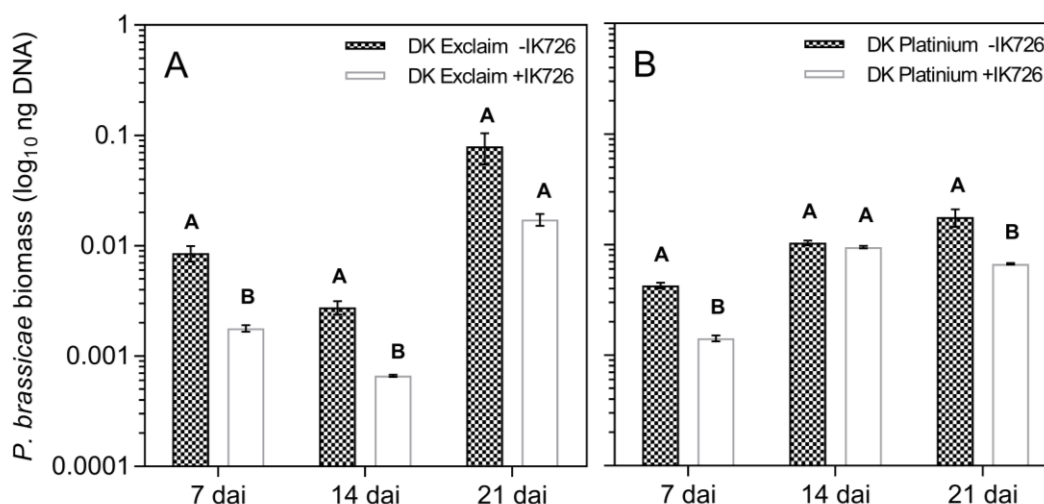


Figure 2. Quantification of *P. brassicae* biomass (DNA) in oilseed rape roots using RT-qPCR at 7, 14 and 21 dai. A) DK-Exclaim, B) DK Platinum. Seeds of each cultivar were either coated with *C. rosea* (marked IK726) or treated with water (control) and subsequently grown in medium inoculated with *P. brassicae*. The experiment was repeated 3 times and data are from individual experiments. Means within each cultivar marked with different letters are significantly different. Error bars represent standard error of the mean.

Visual disease assessments and qPCR results for pathogen biomass clearly show that seed coating with *Clonostachys rosea*, isolate IK276, can significantly reduce the infection of oilseed rape by *Plasmodiophora brassicae*. Not only was severity of infection reduced, but also the incidence was lowered and thus, IK726 can have a two-fold effect on the pathogen, which is quite promising from a disease control perspective. Furthermore, the reductions were shown in both a susceptible cultivar as well as a cultivar where resistance was eroded, thus demonstrating that the effects are quite robust. In greenhouse trials, Peng *et al.* (2011), also found reductions in disease severity index of clubroot when oilseed rape seeds were coated with the product Prestop (containing *C. rosea*), but only at reduced inoculum levels in

infested field soil and in soil-less potting mix inoculated with resting spores of the pathogen. In contrast, IK726 reduced clubroot symptom development in the susceptible cultivar even when the disease incidence was 100%.

The visual reductions in disease severity and incidence were supported by the qPCR data, which showed less biomass of *P. brassicae* when plants were raised from seeds coated with IK726. Differences in pathogen biomass were already seen as early as 7 dai, as also observed by Lahlali & Peng (2014), where there are no visible symptoms. Such an early reduction indicates that IK726 reduces the initial infection by the pathogen and this would be very impotent epidemiologically since it will prevent the pathogen from being established and damage the plant, potentially leading to less inoculum production.

In conclusion, it is clear that seed coating with *C. rosea*, isolate IK276, has promising possibilities for biological control of *P. brassicae*, also in an IPM context. Thus, with a fairly low amount of BCA, disease was controlled at both high and low infection levels and also in plants where the inherent genetic disease resistance is no longer effective. Further work includes optimisation of the qPCR biomass quantification, investigations of the mechanisms by which IK726 reduce disease when used as a seed coating as well as field trials to further explore the feasibility of using IK726 for clubroot disease control.

## Acknowledgements

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## Effects of calcium cyanamide, burnt lime and cultivar resistance on suppression of clubroot disease in oilseed rape cultivation

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**Abstract:** Clubroot, caused by *Plasmodiophora brassicae*, is an internationally important disease of oilseed rape (*Brassica napus*), causing serious losses in Europe, North America and Australia. Nowadays, the disease is an increasing problem not only to oilseed rape but also to all Brassica species. The detection of 124 new *P. brassicae*-infested fields during 2013-2017 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 (Zamani-Noor, 2017). At the present study, calcium cyanamide and burnt lime used with cultivar resistance were evaluated for their potential in integrated management of clubroot disease in oilseed rape cultivation. Multifactorial field trials with natural infection were conducted on three different locations in Germany in 2014, 2015 and 2016. The plots consisted of two winter oilseed rape cultivars differing in their levels of resistance to clubroot and subplots of two soil amendments which were applied at different time points. Calcium cyanamide (300 kg/ha; 50% calcium oxide) and burnt lime (1500 kg/ha) were distributed evenly 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. The results showed that the incidence and severity of clubroot disease varied across locations and years. The most severe disease was observed in all locations in 2014 in which the clubroot-resistant oilseed rape cv. Mendel also showed strong infection in one field. In other two fields, the resistant cultivar provided up to 90% disease control. A slight increase in soil pH, about 0.2-0.5 unit higher than the natural soil suspension pH, was observed after application of calcium cyanamide or burnt lime. However, a few days later the soil pH decreased again and was as equal as control plots. Changing the time of fertilizer's 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 fertilizers at later growth stages. In comparison with calcium cyanamide (up to 30% disease control), burnt lime application has a smaller effect. In 2014, nearly half yield losses were recorded in susceptible cultivar in controlled plots in compare to the treated ones. The clubroot disease pressure was considerably lower in 2015 and 2016, probably due to the unfavourable weather conditions for the pathogen. 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 and severity of the disease.

**Key words:** *Brassica napus*, *Plasmodiophora brassicae*, pathotype, calcium cyanamide, burnt lime, disease severity



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## **Impact of mepiquat chloride on root development system in oilseed rape**

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**Abstract:** The root system is vital for plants to grow and develop well. Promoting the initiation of roots will contribute to enhancing the resistance of plants to biotic and abiotic stress. Plant growth regulators as mepiquat chloride (MC) are organic substances that modify the course of physiological processes in plants. The effects of MC application to oilseed rape cv. Bellevue and ES Cesario F1 on the initial growth of the roots were studied in a chamber with controlled environmental conditions. Measurements of total root length, average root diameter and surface area were made using the Smart Root programme. MC had a positive effect on all studied parameters. In comparison to controls, it increased the thickness of the root neck (11% for Bellevue, 13% for ES Cesario F1) as well as the length (4% for Bellevue, 6% for ES Cesario F1), total length (64% for Bellevue, 83% for ES Cesario F1) and volume (68% Bellevue, 86% for ES Cesario F1) of the root system.

**Key words:** mepiquat chloride, growth retardant, roots, oilseed rape

### **Introduction**

Manipulation of plant architecture using growth regulators can be an agronomic strategy for improvement of winter hardiness, limitation of lodging and improvement of yield by increasing the number of branches, increasing the efficiency of the root system and better anchoring of the plant in the soil (Daniels *et al.*, 1982). Plant growth regulators are organic substances other than nutrients that modify the course of physiological processes in plants (Nickell, 1979). Growth regulators work inside plant cells where they stimulate or inhibit specific hormones involved in metabolic processes (Spitzer *et al.*, 2011). Mepiquat chloride, 1,1-dimethylpiperidin chloride (MC), is a synthetic growth retardant used to control plant height and indirectly to modify the rooting pattern because it acts in the partition of biomass, inhibiting the growth of some parts while enhancing others. The objective of the present experiment was to study the effects of MC on root development in oilseed rape.

### **Material and methods**

Washed roots of oilseed rape (*Brassica napus* L.) cv. Bellevue (Saaten-Union Poland) and ES Cesario F1 (Euralis, Poland) were spread onto a piece of blotting paper and kept in vials in a 3% solution of formaldehyde in a refrigerator for later analyses. Debris and dead roots were manually removed from living roots based on their color and flexibility. Prior to imaging, the roots were washed and drained thoroughly. In order to improve the image contrast and to

facilitate the choice of a proper threshold value for scanning, roots were then stained. They were placed in a warm (40 °C) solution of Azur-Eosin-Methylenblue Giemza (diluted 1:25 with distilled water) for 10 min. Stained roots were gently rinsed for at least 3 min under running water, carefully spread in a thin layer of water (2-3 mm) on a transparent slide and scanned. Measurements, made using the Smart Root programme, involved total root length, average root diameter and surface area.

## Results and discussion

Mepiquat chloride increased all studied parameters of root system development in oilseed rape. In comparison to control, it increased the thickness of the root neck (11% for Bellevue, 13% for ES Cesario F1) as well as the length (4% for Bellevue, 6% for ES Cesario F1), total length (64% for Bellevue, 83% for ES Cesario F1) and volume (68% for Bellevue, 86% for ES Cesario F1) of the root system (Figure 1).



Figure 1. The comparison of roots systems of oilseed rape plants treated (upper part) and not treated (lower part, control) by mepiquat chloride (concentration 0.35% the equivalent of 0.7 l/ha).

Mepiquat chloride may contribute to better uptake of water, mineral salts and use of solar energy, and consequently affect the process of yield formation (Ramachandra-Reddy *et al.*, 1996). It can reduce the effects of drought and improve the regeneration of the root system after damage.

As reported by Nickell (1979), the response of plants to growth regulators may vary between varieties. It means, that a certain plant growth regulator may affect cultivars in a different way, depending on their earliness, above-ground and below-ground plant architecture, stage of development, plant fertilization/nutrition scheme, the stage of plant

physiology and also depending on environmental conditions. However, more research is needed in respect to the studies of the effect of artificially synthesized plant growth regulators on various cultivars of oilseed rape. Moreover, it would be interesting to learn their effect on plant-pathogen and plant-soil microbe interactions.

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**Pathology Session 2:  
Quantitative and qualitative resistance  
to stem canker**



## Effects of three fungicides on *Leptosphaeria maculans* isolates collected in the Czech Republic

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### Introduction

Blackleg disease or phoma stem canker is a major yield limiting factor to the world's third most important oil crop, oilseed rape. It is caused by *Leptosphaeria maculans* (Ces & de Not) (anamorph: *Phoma lingam*) and *L. biglobosa*. In the Czech Republic, a survey of oilseed rape producing areas between 2007 and 2009 showed the prevalence of both *L. maculans* and *L. biglobosa* (Rysanek *et al.*, 2010). Worldwide, Blackleg disease is responsible for losses especially in Canada, Europe and Australia, with losses recorded up to £ 1 billion (Fitt *et al.*, 2011). As a result, it has become necessary to cut down these losses to increase productivity and yield. Because the inoculum can survive as a saprophyte for up to 4 years, making it a good inoculum source, some cultural control methods such as stubble removal and a good crop rotation system have been used. Other control methods such as chemical and genetic resistance have also been known to curb this disease. Chemical control measures include the use of seed treatments, which are effective in limiting the survival of seed borne inoculum in infected plants. Others such as foliar fungicides and soil fungicides are also used to control *L. maculans*. In Australia and Canada, because of the low potential yield in these parts, seed treatment and soil fungicides are the major chemical control methods. In Western Europe however, foliar fungicides are used in addition to seed treatments and soil fungicides as these areas record high yield. What is important however is these chemicals are applied timely, generally in autumn, soon after the appearance of phoma leaf spots (West *et al.*, 2001). The Ergosterol Biosynthesis Inhibitors (EBI), mainly the triazoles and Methyl Benzimidazole Carbamates (MBC) like Benomyl and carbendazim are the major classes of foliar fungicides used. Fungicides, especially those that are site specific, become resistant after continuous use, thereby reducing their efficacy and lifespan. In Eckert *et al.* (2010), *in vitro* studies showed that mycelial growth of *L. maculans* isolates are sensitive to triazole fungicides like flusilazole and tebuconazole. However, the effects of fungicides on individual single spore *L. maculans* isolates on other EBIs and MBCs have not been carried out. It is important to know which fungicides are sensitive or resistant to the individual *L. maculans* isolates to prolong their effectiveness. It is therefore necessary to monitor commonly used fungicides to know how effective they are and also gain insight into potential resistance problems. The objective therefore was to determine the efficacy and level of sensitivity of different fungicides against individual *Leptosphaeria maculans* isolates.

## Material and methods

About 129 leaf samples of oilseed rape cultivars with *L. maculans* or *L. biglobosa* symptoms have been collected in 45 oilseed rape producing areas in the Czech Republic. Five hundred single spore pycnidium isolates have been obtained by cutting out the phoma lesions and then creating a moisture chamber using moist filter paper. Using a microscope, three single pycnidia were isolated from each phoma leaf spot. They were maintained on either PDA or V8 media at 20 °C in the dark.

Multiplex PCR was used for differentiating between the *L. maculans* isolates and the *L. biglobosa* isolates (Liu *et al.*, 2006).

Using the agar-plate assay method, fungicides were mixed into the V8 agar at different concentrations (0, 0.001, 0.01, 0.1, 1.0, and 10) µg/ml. With a cork borer, each *L. maculans* isolate was placed on Petri dishes amended with the fungicides at the different concentrations. These were placed in the dark at 20 °C for 14 days. Fungicides used include Tetraconazole, Boscalid and Trifloxystrobin. The average fungal growth inhibition rate, radial measurements were taken and EC<sub>50</sub> values of fungicides of each isolate was calculated.

## Results and conclusions

Preliminary results showed that generally, EC<sub>50</sub> values ranged from 0.09 µg/ml to 66.13 µg/ml. There were marked differences between the sensitivities of the fungicides. Tetraconazole was the least sensitive fungicide with EC<sub>50</sub> values between 0.8 µg/ml and 66 µg/ml while Boscalid was the most sensitive with EC<sub>50</sub> value between 0.09 µg/ml and 0.49 µg/ml. Trifloxystrobin however had an EC<sub>50</sub> value range of between 0.26 µg/ml and 12.40 µg/ml. Some isolates amended with the same fungicide (Tetraconazole) also showed different sensitivity levels. These results show that resistance of some *L. maculans* isolates may have already begun with Tetraconazole.

## Acknowledgements

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## **PhomaDur – Improvement of quantitative resistance to stem canker in oilseed rape**

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**Abstract:** *Leptosphaeria maculans* is one of the most important pathogens on oilseed rape. Using resistant cultivars proved to be the best method to manage *L. maculans*. Two types of resistance are generally described, R-gene mediated resistance and quantitative resistance. Quantitative resistance is known to be more durable and stable. PhomaDur is a project that aims to improve quantitative resistance and to deepen our understanding of its mechanisms in oilseed rape. Therefore, the objectives of this project are (1) to phenotype a multi parents' interconnected mapping population in the field and in the greenhouse in order to identify QTLs, (2) to study blackleg infestation and characterize the range of *Phoma lingam* isolates/races in different regions in Germany, and (3) to investigate the quantitative resistance mechanisms. This short abstract paper presents in brief preliminary results of the ongoing project.

**Key words:** *Leptosphaeria maculans*, quantitative resistance, *Phoma lingam*, races, oilseed rape, *Brassica napus*

### **Introduction**

Blackleg is a disease of economic importance in oilseed rape production. To limit the yield losses caused by *L. maculans*, resistant cultivars are the milestone of the breeders' and phytopathologists' efforts. Major gene resistance is mostly overcome within few years after release of a new cultivar due to the evolution of virulent *L. maculans* isolates. Quantitative resistance is a polygenic race-nonspecific resistance. Thus, it is more robust. Recent studies showed that combining R genes and quantitative resistance increases the effectiveness of cultivar resistance in oilseed rape (Huang *et al.*, 2018). This paper compares disease incidence and disease severity at four sites in Germany in the growing season 2017-2018. Second, it presents preliminary results on the *Phoma lingam* race distribution in German fields. Third, it discusses quantitative resistance based on different inoculation methods on different plant tissues.

### **Material and methods**

Field experiments were established in four regions in Germany, namely Hadmersleben, Einbeck, Groß Helle and Nienstädt in autumn 2017. Samples were collected in October 2017 and May 2018 to evaluate the progress of the disease during the season. Disease incidence and disease severity were defined based on the leaf spots in autumn and on both leaf spots and stem canker in spring. NK Bravour was used as a trap cultivar to isolate *Phoma lingam*. To

describe *L. maculans* race spectrum, two differential sets were used; a French differential set that includes *B. napus* genotypes harboring the major resistance genes *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm7* and *Rlm9* (Winter & Koopmann, 2016), and a Canadian differential set consisting of different Topas Lines, each harboring one of the following major genes *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *LepR1*, *LepR2*, *LepR3* (Larkan *et al.*, 2016). Cotyledon test was conducted to characterize *L. maculans* races.

In the greenhouse, a study aiming to evaluate quantitative resistance against *L. maculans* in *B. napus* based on inoculation at different plant tissues was conducted. Seven varieties were inoculated using four different inoculation methods. The plants were inoculated either by spraying with spore suspension  $10^7$  spores/ml on the (1) leaf upper side or (2) leaf underside or by mycelial agar plug application (3) directly on the petiole or (4) at the stem base. Disease phenotyping was applied based on a scale from 1-9 based on a modified key from Kutcher *et al.* (1993) where the volumes of diseased tissues are calculated.

## Results and discussion

The infestation of *L. maculans* during the season 2017-2018 was higher than 85% in Hadmersleben, Einbeck, Groß Helle and in Nienstädt. However, Nienstädt showed the highest disease incidence and disease severity in autumn and spring. Until now, 8 races were described in Hadmersleben in Germany. Most isolates showed avirulent reactions with varieties harboring *Rlm7*, *LepR1* or *LepR2*. Consistent with an earlier study (Winter & Koopmann, 2016), four different *Rlm7*-resistance breaking races were found.

The infection pathway of the hemibiotroph *L. maculans* goes through different plant tissues. This study aims to investigate whether quantitative resistance mechanisms cease the pathogen development even before *L. maculans* reaches the stem bases. The tested cultivars showed different reactions to inoculation with *L. maculans*. Under controlled conditions, results show that the varieties reacted differently based on the inoculum placement. By spraying spore suspension on the lower side of the leaves, one variety showed to be more susceptible than the susceptible check. However, inoculation by agar plug on either the stem or the petiole induced the highest disease severity on the susceptible check compared to the other varieties. Other cultivars used in this experiment were consistently classified resistant regardless of the applied inoculation method. The isolate used in this study was examined on cotyledons and expressed virulence on any of the tested varieties. Thus, the differences between the varieties are likely attributed to quantitative resistance in different plant tissues rather than to monogenic qualitative resistance.

## Acknowledgements

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## Screening and identification of resistance to stem canker (*Leptosphaeria* spp.) and downy mildew (*Hyaloperonospora brassicae*) in *Brassica* hybrids

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**Abstract:** The aim of this study was to identify the sources of genetic resistance to stem canker and downy mildew in *Brassica* hybrids obtained from the crossings between five high yielding cultivars (Californium, Lisek, Jet Neuf, Górczański, Skrzyszowicki) of *B. napus* and eight *Brassica* species. The experiment was done in field and under controlled conditions using F<sub>2</sub> and F<sub>3</sub> generations of these interspecific hybrids. Moreover, the determination of *Leptosphaeria* species was studied using Loop-mediated DNA Amplification (LAMP) method. The genotypes differed with their reaction to the pathogens. Forms with increased resistance to blackleg especially among *B. napus* × *B. carinata* and *B. napus* × *B. fruticulosa* hybrids have been found. It was possible to select *Brassica* forms with lower susceptibility to downy mildew.

**Key words:** blackleg, stem canker, downy mildew, oilseed rape, *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, *Hyaloperonospora brassicae*, Loop-mediated isothermal amplification

### Introduction

Stem canker of brassicas (blackleg), caused by the fungal complex *Leptosphaeria maculans* and *L. biglobosa* and downy mildew (*Hyaloperonospora brassicae*, previously referred to as *H. parasitica* and *Peronospora parasitica*) are damaging diseases of oilseed rape worldwide, including Poland (Kaczmarek *et al.*, 2014; Mohammed *et al.*, 2017). High profitability of oilseed rape production has driven great development of breeding of new varieties with new characteristics, including resistance to some diseases in different genetic backgrounds. Every year, new population and hybrid varieties are introduced to the market. The aim of this study was to characterize the resistance of F<sub>2</sub> and F<sub>3</sub> *Brassica* hybrids to downy mildew and stem canker in field conditions. In order to evaluate the relative impact of either fungal species, the *Leptosphaeria maculans* – *L. biglobosa* complex has been studied using the LAMP technique.

### Material and methods

Screening of plant susceptibility/resistance was done in the field and under controlled conditions. The field experiment was done in Wielkopolska (Greater Poland) region, 90 km westwards from its capital (Poznań). Twenty five cross combinations of F<sub>2</sub> and F<sub>3</sub> *Brassica* hybrids, obtained via interspecific hybridization were analyzed:

1. *B. napus* cv. Californium × *B. rapa* ssp. *trilocularis*
2. *B. napus* cv. Californium × *Sinapis alba*
3. *B. napus* cv. Lisek × *B. fruticulosa*
4. *B. napus* cv. Lisek × *B. carinata*
5. *B. napus* cv. Californium × *B. rapa* ssp. *chinensis*
6. *B. napus* cv. Californium × *B. juncea* acc. 633007
7. *B. napus* cv. Californium × *B. juncea* cv. Ames
8. *B. napus* cv. Californium × *B. juncea* cv. Ames
9. *B. napus* cv. Californium × *B. nigra*
10. *B. napus* cv. Californium × *B. juncea* cv. Ames
11. *B. napus* cv. Jet Neuf × *B. rapa* ssp. *pekinensis*
12. *B. napus* cv. Górczański × *B. rapa* ssp. *pekinensis* acc.08007574
13. *B. napus* cv. Lisek × *B. tournefortii*
14. *B. napus* cv. Górczański × *B. rapa* Pak Choi
15. *B. napus* cv. Californium × *B. oleracea* ssp. *alboglabra*
16. *B. napus* cv. Californium × *B. rapa* ssp. *pekinensis* acc.08006169
17. *B. napus* cv. Skrzyszowicki × *B. rapa* ssp. *pekinensis* 08006169
18. *B. napus* cv. Górczański × *B. rapa* ssp. *pekinensis* 08006169
19. *B. napus* cv. Lisek × *B. rapa* ssp. *pekinensis* acc. 08006169
20. *B. napus* cv. Californium × *B. fruticulosa* acc. PI649097
21. *B. napus* cv. Californium × *S. alba* cv. Bamberka
22. *B. napus* cv. Californium × *B. fruticulosa* acc. PI649102
23. *B. napus* cv. Jet Neuf × *B. carinata* acc.PI649094
24. *B. napus* cv. Jet Neuf × *B. carinata* cv. Dodolla
25. *B. napus* MS8 × *B. carinata* acc. PI 649099

Two-week old seedlings of the differential set of hybrids were inoculated with four isolates of *L. maculans* with known avirulence genes and a field population of *Hyaloperonospora brassicae*. For the cotyledon test using *L. maculans* the plants were grown in plastic trays in an temperature controlled chamber, maintained at 20-22 °C day/ 16-18 °C night temperatures with a 12 h light/12 h dark photoperiod. Droplets of spore suspensions were placed on half of a cotyledon that had been wounded by puncturing with a sterile needle prior inoculation. Host responses were scored 14, 21 and 28 dpi, using a 0-6 scale of disease severity. The population of *Leptosphaeria* was also studied using Loop-mediated DNA amplification (LAMP) method. For this purpose leaf or stem samples were collected from ten individual plants per genotype. The inoculation with *H. brassicae* was performed at the stage of 6-8 true leaves, in three replicates of 6 plants each. Inoculum in the form of the leaves of oilseed rape infected with the pathogen was evenly distributed to all pots and spread around them. The plants were kept in glasshouse conditions with 20-22 °C day/16-18 °C night temperatures and 12 h light/12 h dark regime. The disease scoring was done 4 weeks after inoculation, using a 0-9 scale.

## Results

The hybrids differed with their reaction to stem canker. In both seasons forms with increased resistance to blackleg especially among *B. napus* × *B. carinata* and *B. napus* × *B. fruticulosa* hybrids have been found. In field conditions most of the isolates found in the autumn (72%) were identified as *L. maculans*, whereas before harvest the stems were infected with both species, with marginal predominance of *L. biglobosa* (56%). There were no *H. brassicae* symptoms observed on tested plants in field conditions.

In the controlled conditions statistically significant differences were found following the inoculation with *H. brassicae*, with the hybrids of *B. napus* cv. Californium × *S. alba* and *B. napus* cv. Jet Neuf × *B. rapa* ssp. *pekinensis* among the most resistant ones. In general the symptoms of downy mildew were mild to intermediate (Figure 1).

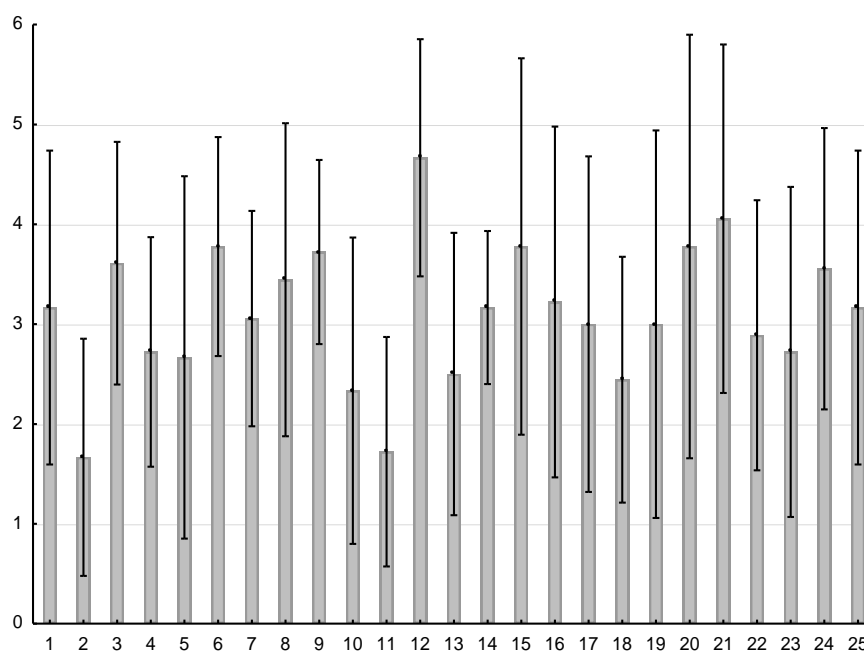


Figure 1. The distribution of the symptoms of downy mildew (Y axis: disease scoring 0-9, where 0 is healthy) among twenty-five cross variants of F<sub>2</sub> and F<sub>3</sub> *Brassica* hybrids (see the list in Materials and methods), obtained *via* interspecific hybridization.

## Conclusions

1. The hybrids of *Brassica* differ in their reaction to *Leptosphaeria* spp., which makes it possible to select forms with higher resistance to stem canker.
2. In the spring the species *L. maculans* dominates in field conditions over *L. biglobosa*, but it is slightly overtaken by *L. biglobosa* in the summer, which reflects changes in the composition of pathogen population of fungi causing blackleg on oilseed rape in Poland.
3. It is possible to select *Brassica* forms with lower susceptibility to downy mildew.

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## Prediction of ascospore release of *Leptosphaeria* spp. to improve timing of fungicide applications

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**Abstract:** Temperature and rainfall influence the timing of release of ascospores of *Leptosphaeria* spp., which are the initial source of inoculum causing the disease phoma stem canker on oilseed rape. Phoma stem canker of oilseed rape is controlled chemically by the application of fungicide seed treatments or foliar fungicide sprays. A forecast system for phoma stem canker has been developed (<http://www.rothamsted.ac.uk/phoma-leaf-spot-forecast/phoma-forecast>) for guiding timing of fungicide sprays. This forecast provides information on the date in autumn when 10% of oilseed rape plants in a crop are expected to show symptoms of phoma leaf spot, which is generally the threshold for a fungicide application against phoma stem canker. The forecast uses temperature and rainfall data to simulate the development of the pathogen on crop debris and subsequent infection of young plants. The model described by Huang *et al.* (2007) was used to predict the first dates when 10% of the maximum ascospore release occurred at three sites in the UK in the 2010/11, 2011/12 and 2012/13 cropping seasons and at one site in the 2013/14 cropping season. The environmental factors at each site in each cropping season that may have influenced these differences are discussed. Moreover, this study examined the relationships between accumulated rainfall (mm) for the period from the date of 25% of maximum ascospore release until the date of maximum ascospore release and the date of maximum ascospore release. There is evidence that this parameter could be used to model the date of maximum of ascospore release to guide the timing of fungicide treatments.

**Key words:** Phoma stem canker, oilseed rape, ascospore release

### Introduction

Phoma stem canker (*Leptosphaeria maculans*) is an important disease on oilseed rape (*Brassica napus*) in the UK, causing losses to farmers of > £ 100 M p. a., despite the use of fungicides costing £ 20 M p. a. (Fitt *et al.*, 2006 a; Stonard *et al.*, 2010). Oilseed rape cultivation is of vital importance for the UK industry, benefiting both the export market for biodiesel and vegetable oil and the farming industry as a break crop in a cereal rotation.

The temperature and rainfall for the period between the harvest of the previous crop and the establishment of the new crop are crucial in determining timing of phoma leaf spot epidemics, because they affect both the maturation of pseudothecia (fruiting bodies) of *L. maculans* on crop debris providing ascospores (sexual spores) and the infection of leaves that occurs through stomata (Evans *et al.*, 2010). The ascospores can travel from several hundred metres to several hundred kilometres (Travadon *et al.*, 2011) and act as initial



inoculum before the production of conidia (asexual spores) at the site of infection (Rouxel and Balesdent, 2005). Climate models have predicted that global warming will increase the UK range and severity of phoma stem canker epidemics (Evans *et al.*, 2008; Butterworth *et al.*, 2010).

Severe disease is associated with deep girdling stem cankers; they restrict the flow of water and nutrients to the developing seed before harvest, causing premature ripening, shriveled seeds and seed pods that result in substantial yield losses (Fitt *et al.*, 2006 b). A forecast system for phoma stem canker has been developed: (<http://www.rothamsted.ac.uk/phoma-leaf-spot-forecast/phoma-forecast>). This forecast provides information on the date in autumn when 10% of oilseed rape plants are expected to show symptoms of phoma leaf spot, which is generally the threshold for a fungicide application against the disease. The forecast uses temperature and rainfall to simulate the development of the pathogen on crop debris and subsequent infection of young plants.

This study used weather and ascospore release data to predict the first dates when 10% of the maximum ascospore release occurred and gave insights for optimisations to model data to predict the date of maximum of ascospore release to guide the timing of fungicide treatments.

## Material and methods

### *Prediction of the first date of 10% of the maximum ascospore release*

Previous work had shown that there was a linear relationship between the first date when 10% of the maximum ascospore release occurred and the dates when 30% of pseudothecia were mature (Huang *et al.*, 2007). This relationship was used to predict the first dates when 10% of the maximum ascospore release occurred at three sites (Bayfordbury, Cowlinge and Rothwell) in the 2010/11, 2011/12 and 2012/13 cropping seasons and at one site (Cowlinge) in the 2013/14 cropping season.

### *Examining the influence of accumulated rainfall on ascospore release dates*

The weather and ascospore release data at three sites in the 2010/11, 2011/12 and 2012/13 cropping seasons and at one site in the 2013/14 cropping season were used. Relationships were fitted between the number of days from the date of 25% of maximum ascospore release ( $d_{25max}$ ) until the date of maximum ascospore release ( $d_{max}$ ) and the accumulated rainfall (mm) ( $F$ ) from the 1 August or from the date of 25% of maximum ascospore release until the date of maximum ascospore release. The percentage of variation accounted for ( $R^2$ ) was calculated by fitting the following simple linear relation:

$$F = a (d_{max} - d_{25max}) + b \quad \text{Equation 1}$$

Relationships were made between accumulated rainfall (mm) ( $F$ ) from the date of 25% of maximum ascospore release until the date of maximum ascospore release and the date of maximum ascospore release ( $d_{max}$ ). The percentage of variation accounted for ( $R^2$ ) was calculated by fitting the following relation:

$$F = a (d_{max}) + b \quad \text{Equation 2}$$

## Results and discussion

### Prediction of the first date of 10% of the maximum ascospore release

Monitoring of the numbers of air-borne ascospores of *Leptosphaeria* species by Burkard volumetric spore samplers at Cowlinge in the 2010/11 cropping season started on 13 October 2010 and since numbers of ascospores observed were already 17% of maximum ascospore release, the date of 10% maximum ascospore release was estimated as 30 September 2010 are shown in Table 1. Monitoring of the numbers of air-borne ascospores of *Leptosphaeria* species by Burkard volumetric spore samplers at Cowlinge in the 2012/13 cropping season started on 26 September 2012. Since either no ascospores or < 6% of maximum ascospore release were observed for 25 days after the beginning of ascospore sampling, the observed date of 10% maximum ascospore release in this season (20 October 2012) was used for the regression analysis.

Table 1. Observed and predicted first dates of 10% of the maximum ascospore release of *Leptosphaeria* species at three sites in the 2010/11, 2011/12 and 2012/13 cropping seasons and at one site in the 2013/14 cropping season.

Cropping season	Site	Date 10% maximum ascospore release	
		Observed <sup>a</sup>	Predicted <sup>b</sup>
2010/11	Bayfordbury	19 September 2010	06 September 2010
	Cowlinge	(30 September 2010) <sup>c</sup>	03 September 2010
	Rothwell	14 October 2010	6 September 2010
2011/12	Bayfordbury	04 November 2011	05 September 2011
	Cowlinge	19 November 2011	11 September 2011
	Rothwell	24 November 2011	20 September 2011
2012/13	Bayfordbury	26 October 2012	15 September 2012
	Cowlinge	(20 October 2012) <sup>d</sup>	29 August 2012
	Rothwell	30 October 2012	12 September 2012
2013/14	Bayfordbury	N/A <sup>e</sup>	N/A
	Cowlinge	20 October 2013	19 September 2013
	Rothwell	N/A	N/A

<sup>a</sup> The 10% of maximum ascospore release values were calculated from the maximum ascospore data at each site for each cropping season

<sup>b</sup> The predicted date of 10% maximum ascospore release was calculated, based on weather factors, as described in Huang *et al.* (2007)

<sup>c</sup> Ascospore sampling started on 13 October 2010 at Cowlinge and in parentheses is the estimated first date of 10% maximum ascospore release

<sup>d</sup> Ascospore sampling started on 26 September 2012 at Cowlinge and in parentheses is the estimated first date of 10% maximum ascospore release

<sup>e</sup> No weather and ascospore data were obtained at Bayfordbury and Rothwell in the 2013/14 cropping season

The fitted lines between the observed first date when 10% of the maximum ascospore release occurred ( $m_{10o}$ ) and the predicted first date when 10% of the maximum ascospore release occurred ( $m_{10p}$ ) are shown in Figure 1. In Figure 1a the relationships were fitted by linear regression of first date when 10% of the maximum ascospore release was observed ( $m_{10o}$ ) on 10% of the maximum ascospore release predicted ( $m_{10p}$ ), including the estimated dates for Cowlinge in the 2010/11 and 2012/13 cropping seasons ( $m_{10o} = 0.15 m_{10p} - 68.6$ ;  $R^2 = 0.25$ ) or in Figure 1b excluding them ( $m_{10o} = 1.63 m_{10p} - 116.5$ ,  $R^2 = 0.21$ ). The small value of  $R^2$  suggested that there was no great potential for using that model to forecast the date of the first major ascospore release for these data sets. However, a greater number of data points including data sets from cropping seasons with large variations in environmental conditions would be needed to validate this model precisely and provide insights for optimisations.

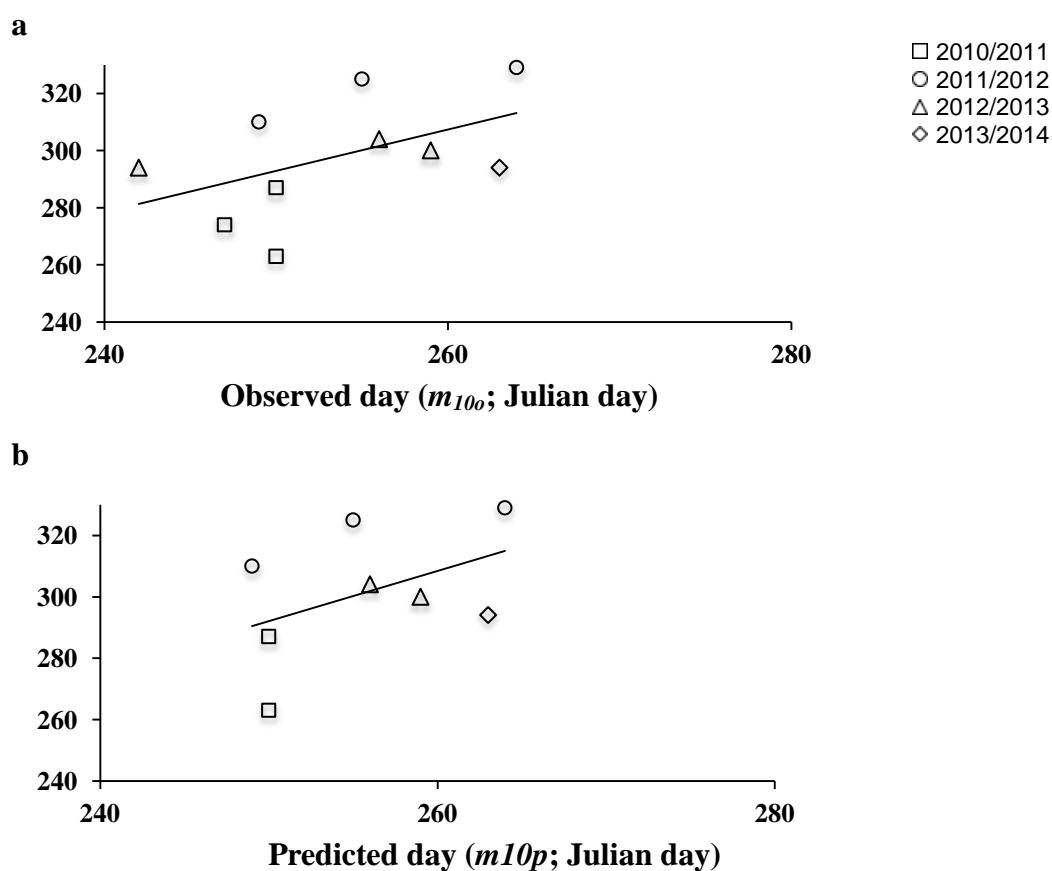


Figure 1. Relationships between observed first date (Julian day) when 10% of the maximum number of ascospores (collected by a Burkard spore sampler) were released and predicted first date (Julian day) when 10% of the maximum number of ascospores were released with (a) and without (b) the estimated dates for Cowlinge in the 2010/11 and 2012/13 cropping seasons.

When the model described by Huang *et al.* (2007) was applied to predict the first date when 10% of the maximum ascospore release occurred, the predicted first dates when 10% of the maximum ascospore release occurred were earlier than the dates when 10% of the maximum ascospore release were observed. The mean difference between observed and

predicted first dates when 10% of the maximum ascospore release occurred (residual) was 45 days as shown in Figure 2. All the residuals were positive and indicated that all predicted dates were earlier and varied between 13 (at Bayfordbury in the 2010/11 cropping season) and 70 days (at Cowlinge in the 2011/12 cropping season). The mean residual for each cropping season was greatest in the 2011/12 cropping season (c. 65 days), followed by that in the 2012/13 cropping season (c. 47 days) and it was least in the 2010/11 cropping season (c. 26 days).

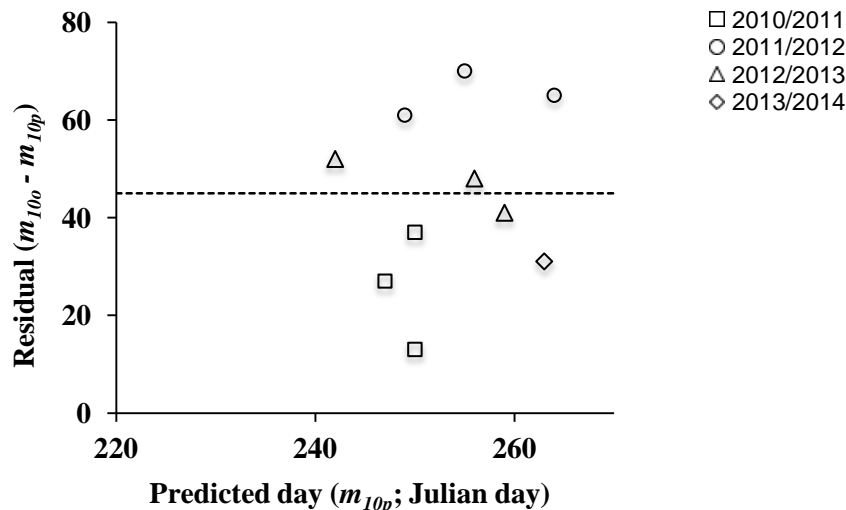


Figure 2. Residuals of the difference between the observed ( $m_{10o}$ ) and the predicted ( $m_{10p}$ ) first dates when 10% of the maximum ascospore release of *Leptosphaeria* species occurred. The dashed line indicates the median value for the residuals (45 days).

The predicted first dates when 10% of the maximum ascospore release occurred for that cropping season were in September, whereas the observed dates when 10% of the maximum ascospore release occurred were in November. The weather in autumn of the 2011/12 cropping season was unusual, with rainfall in August but many dry days in September and October (Figure 3). This may have influenced the rate of pseudothecial maturation, and the subsequent ascospore release, resulting in a later date of maximum ascospore release in 2011/12 compared to other cropping seasons. Previous studies have shown that the stem debris wetness and its duration is an essential factor for pseudothecial maturation to progress (Petrie, 1995; Pérès *et al.*, 1999; Kaczmarek *et al.*, 2011; Piliponytė-Dzikienė *et al.*, 2014; Sidique, 2016).

#### ***Examining the influence of accumulated rainfall on ascospore release dates***

The number of days from the date of 25% of maximum ascospore release ( $d_{25max}$ ) until the date of maximum ascospore release ( $d_{max}$ ) was related to the accumulated rainfall (mm) from the 1 August until the date of maximum ascospore release are shown in Figure 4 a or the date of 25% of maximum ascospore release until the date of maximum ascospore release are shown in Figure 4 b. The best fitted lines were  $F = 2.04 (d_{max} - d_{25max}) + 165.5$  ( $R^2 = 0.64$ ) or  $F = 1.81 (d_{max} - d_{25max}) + 6.7$  ( $R^2 = 0.75$ ), respectively.

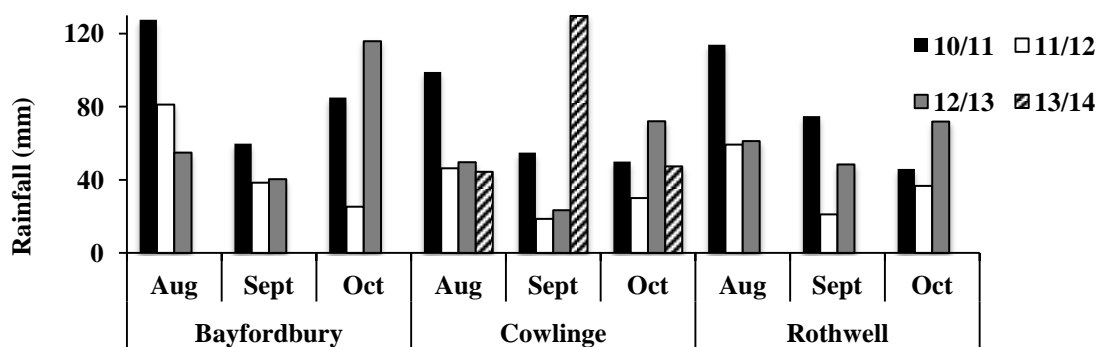


Figure 3. Total rainfall (mm) in the months of August, September and October at three sites in the 2010/11, 2011/12 and 2012/13 cropping seasons and at one site in the 2013/14 cropping season.

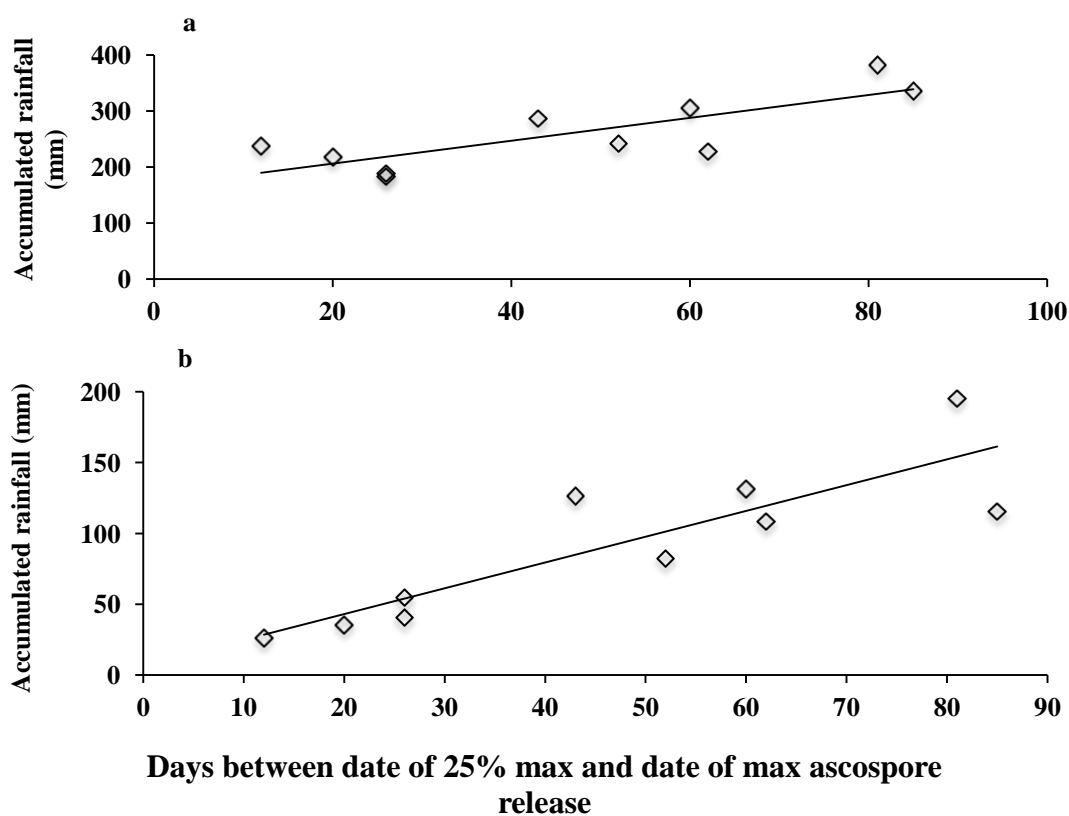


Figure 4. Relationships between accumulated rainfall and the number of days from the date of 25% of maximum ascospore release until the date of maximum ascospore release. The relationships between the number of days from the date of 25% of maximum ascospore release until the date of maximum ascospore release and the accumulated rainfall (mm) for the period (a) from the 1 August until the date of maximum ascospore release or (b) from the date of 25% of maximum ascospore release until the date of maximum ascospore release. The best fitted lines are  $F = 2.04 (d_{max} - d_{25max}) + 165.5$  (a) or  $F = 1.81 (d_{max} - d_{25max}) + 6.7$  (b).

It was apparent that there was a good, significant relationship between accumulated rainfall (mm) for the period from the date of 25% of maximum ascospore release until the date of maximum ascospore release and the date of maximum ascospore release ( $R^2 = 0.35$ ). This relationship was improved ( $R^2 = 0.88$ ) when data with extreme values were not included, (e. g. Bayfordbury in the 2010/11 cropping season; a great amount of rain in days with average daily temperature  $> 10$  °C, and Rothwell in the 2012/13 cropping season; great amount of rain in days with average daily temperature  $> 0$  °C, compared to those in the other cropping seasons). This indicates that this parameter could be used to model the date of maximum of ascospore release.

The accumulated rainfall (mm) for the period from the date of 25% of maximum ascospore release until the date of maximum ascospore release was related to the date of maximum ascospore release were calculated and shown in Figure 5. The number of days from the date of 25% of maximum ascospore release until the date of maximum ascospore release occurred was associated with the accumulated rainfall covering that period. The best fitted lines were  $F = 0.77 (d_{max}) + 156$  ( $R^2 = 0.35$ ) in Figure 5 a, or without two outlier data points,  $F = 1.23 (d_{max}) - 359.8$  ( $R^2 = 0.88$ ) in Figure 5 b. The timings of different proportions of ascospore release (10% or 25% of the maximum or the maximum) in the cropping season along with the developmental stage of the crop could be used as components of forecasting schemes to predict the severity of phoma stem canker epidemics before harvest. Predicting the timing of ascospore release could be combined with models that describe phases of phoma stem canker development (Evans *et al.*, 2006; Evans *et al.*, 2008) to forecast the severity of stem canker epidemics at the end of the cropping season to guide timings of cost effective fungicide applications in autumn.

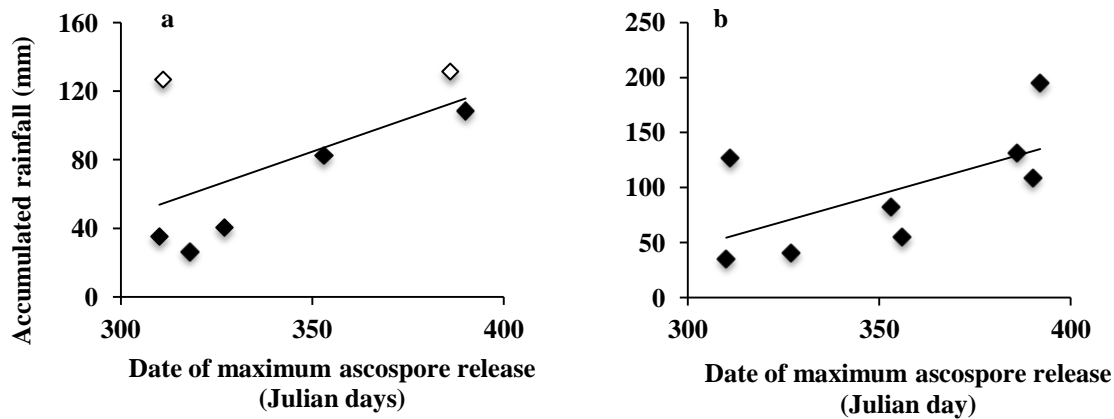


Figure 5. Relationships between accumulated rainfall and the date of maximum ascospore release. The relationships between accumulated rainfall (mm) for the period from the date of 25% of maximum ascospore release until the date of maximum ascospore release and the date of maximum ascospore release. The analysis was done with (a) all the data points (black and white diamonds) or (b) without the outlier data points (white diamonds). The best fitted lines are  $F = 0.77 (d_{max}) + 156$  (a) or  $F = 1.23 (d_{max}) - 359.8$  (b).

## Acknowledgements

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## ***Leptosphaeria* species causing blackleg disease in oilseed rape and *L. maculans* race incidence in the Czech Republic**

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**Abstract:** Provided survey aims to contribute to effective utilisation of current oilseed rape cultivars in the Czech Republic with respect to incidence of *Leptosphaeria maculans* avirulence alleles to prevent selection of new virulent races from *L. maculans* populations. Presented are the first results of an ongoing project focused on the monitoring incidence of *L. biglobosa* and *L. maculans* species and *AvrLm* alleles in *L. maculans* isolates collected in the Czech Republic during 2017-2021.

**Key words:** phoma stem canker, blackleg, oilseed rape, avirulence genes, resistance genes

### **Introduction**

Blackleg represents one of the most serious diseases of oilseed rape in Europe, Northern America, Australia and other countries. Infectious agent of the disease is a species complex *Leptosphaeria maculans* and *Leptosphaeria biglobosa*, inducing phoma stem canker and phoma leaf spot, respectively. The seriousness of the individual species differs in dependence on cultivation locality. In the Czech Republic, the area sown by oilseed rape is steadily increasing, which together with intensive farming represents a serious problem in oilseed rape cultivation. Crop protection relies mostly on resistant cultivars. While no avirulence genes have been reported yet in *L. biglobosa*, numerous ones (*AvrLm*) have been identified in *L. maculans* and some of them were cloned. Their counter-partners in plants (*Rlm*) have been also described. However, durability of a major gene resistance represents a serious issue due to a high evolutionary potential of these sexually reproducing species. The resistance genes in oilseed rape cultivars are quickly overcome by new races of selected in *L. maculans* population resulting in the “boost” and “bust” cycles. Several wide-scale studies on avirulence alleles have been performed in oilseed growing European countries in past (Balesdent *et al.*, 2006; Winter & Koopmann, 2016).

### **Material and methods**

Ninety isolates are collected in the autumn both in commercial and bait cultivars in 2017. Cotyledons and true leaves with necrotic spots with pycnidia are used for isolation of single-spore isolates. To distinguish *L. maculans* from *L. biglobosa*, the pigment production to a

liquid cultivation media and PCR analysis are used (Mazáková *et al.*, 2017). The race spectra avirulence genes are identified using French differential set of cultivars: Columbus (*Rlm1*, *Rlm3*), Bristol (*Rlm2*, *Rlm9*), MT29 (*Rlm1*), 02.22.2.1 (*Rlm3*), Jet Neuf (*Rlm4*), 01.23.2.1 (*Rlm7*), Goeland (*Rlm9*) as published (Winter & Koopman, 2016).

## Results and discussion

Presented are preliminary results of the project aimed at the monitoring of the incidence of *L. biglobosa* and *L. maculans* species and *AvrLm* alleles in *L. maculans* in the Czech Republic during 2017-2021. The first isolates prepared from *L. maculans*-type lesions (originating from 90 leaf spots) were collected from commercial oilseed rape cultivars currently cultivated in the main production areas in the autumn 2017. Single-pycnidia isolates were prepared and tested by species-specific PCR to distinguish *L. maculans* from *L. biglobosa*. 65% of the isolates revealed as *L. maculans* only, the rest showed to be co-infected by *L. biglobosa*. *L. maculans* isolates were tested on a set of differential cultivars to identify avirulence alleles. The isolates appeared virulent to *Rlm1-4*, *Rlm2-4* and *Rlm9*. None of the isolates was virulent to *Rlm7* major resistance gene.

The project will continue in a wider scale using a trap cultivar Westar, which will be sown in 2018, to catch possible other races non-virulent to commercial cultivars.

## Acknowledgements

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## The signalling of fungal treatments of oilseed rape against *Sclerotinia sclerotiorum* and *Phoma lingam*

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**Abstract:** The infection pressure of pathogens *Sclerotinia sclerotiorum* and *Phoma lingam* in the oilseed rape has been monitored at the workplaces in Opava and Šumperk for a long time. The results from 2016 to 2018 confirmed the strong dependence of infection pressure of pathogens on the weather conditions. The lowest contamination of the oilseed rape petals by *S. sclerotiorum* ascospores was recorded in 2018 and the higher contamination of petals and higher infestation of oilseed rape in 2016. In this year, the infestation of oilseed rape by *Sclerotinia* stem rot was rare. Higher captures of ascospores of pathogens *Leptosphaeria maculans*, *L. biglobosa* (asexual stage *Phoma lingam*) were recorded at the both sites in September and October 2017. In mid-September 2017, higher precipitation (96.2 mm) was recorded in Opava. In Šumperk was recorded higher precipitation 92.5 mm in September 2017. The month October was temperately higher than the long-term normal (+10.49 °C). In both locations Šumperk and Opava there was measured higher temperature than the long-term in all autumn months. The infestation of oilseed rape stems before harvest 2018 ranged from 80 to 90 percent. The highest effect of fungicidal treatment combined with insecticidal treatment on the yield and the thousand seed weight values was reached in 2018.

**Key words:** oilseed rape, diseases, signalling, fungicidal treatment

### Methods

#### *Locations*

The study localities near Opava were situated in the mild climate zone of central Europe at an altitude of 250 and 280 m, long-term meteorological data (1981-2010) were the average annual temperature 8.6 °C and the average annual precipitations amount 567.6 mm.

The localities near Šumperk-Rapotín (North Moravia – CZ, central Europe) lay at the altitude 330 m. The long-term meteorological data (1981-2010), the average annual temperature was 7.27 °C and the average annual precipitation was 702.2 mm.

#### *The signalling methods*

The occurrence of *Sclerotinia sclerotiorum* ascospores on oilseed rape inflorescence was evaluated according to the petal test (Morall, Thompson, 1991). The Burkard trap system and microscopic analysis was used for *Leptosphaeria* spp. ascospores monitoring (Jedryczka *et al.*, 2010; Poslušná *et al.*, 2012).

### Field trials

The field parcel trial was conducted with 5 treatments in 4 replicates, parcel/replicate size was 30 m<sup>2</sup>, used oilseed rape variety DK Exlibris. The combinations of insecticidal and fungicidal treatment in year 2017/2018 are described in Table 3.

## Results

### The monitoring of *Sclerotinia sclerotiorum* ascospores

The lowest contamination of the oilseed rape petals by *S. sclerotiorum* ascospores was recorded in 2018 and the higher contamination of petals and higher infestation of oilseed rape in Opava in 2016. In Šumperk there was recorded higher contamination of petals in 2016 and higher infestation of oilseed rape in 2017. In year 2018 the infestation of oilseed rape by pathogen *S. sclerotiorum* was rare.

### The monitoring of *Phoma lingam*

Between the years 2016 and 2017 there were recorded the higher occurrences of *L. maculans* and *L. biglobosa* in autumn 2017. The increased air raid of ascospores was recorded at the end of September 2017 at the site of Opava, on the locality near Šumperk at the turn of September and October (Figure 1). The higher incidence of leaf infestation by disease was recorded in October 2017.

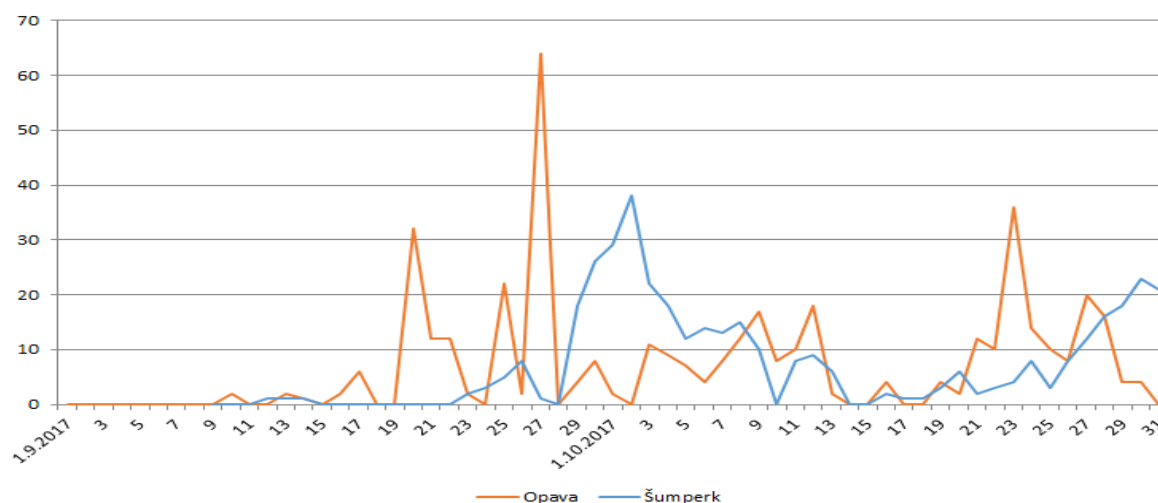


Figure 1. Captures of ascospores pathogen *Leptosphaeria maculans*, *L. biglobosa* (the Burkard trap system)

### Field trials

In 2016/2017 was recorded higher influence of fungicidal treatments on yield than in 2017/2018, when it was observed the higher influence of combined insecticidal and fungicidal treatments of oilseed rape on the yield on the locality Opava. In autumn 2017 there was observed the strong infection pressure of pathogen causing Phoma stem canker (Figure 1) and lower or usual abundance of Coleseed saw-fly (*Athalia rosea*). In the spring 2018 there was observed the high abundance of stem weevils *Ceutorhynchus pallicadctilus* and *C. napi* and at

the end of flowering the medium level of fly activity of Brassica pod midge (*Dasyneura brassicae*). The higher air raid of *S. sclerotiorum* ascospores was recorded at the beginning of flowering (Table 1). The low rainfall in April and May (Table 2) influenced the oilseed rape infestation, which was rated as rare. The high infestation by harmful organisms and the high spring temperatures in combination with the low precipitation in November 2017 to May 2018 (Table 2) caused quick maturation of oilseed rape plants. This was particularly observed on the untreated plots or on plots with low chemical application intensity. The higher intensities of insecticides and fungicides slowed down the quick maturation of crop and increased yield by 33% (Table 3), the yield increase in 2017 was 18%. The harvest in 2018 was 20 days earlier than in the previous year.

Table 1. The air raid of *S. sclerotiorum* ascospores: The contamination of oilseed rape petals (%) and pathogen's infestation (frequency %) before harvest (BBCH 85-87), Opava, Šumperk, 2016-2018, evaluated on the susceptible variety

Location	Beginning of flowering	Full blossom	End of flowering	Infestation before harvest
Opava 2016	55	78	47	69
Opava 2017	15	45	11	28
Opava 2018	42	20	28	Sporadic
Šumperk 2016	28	42	15	40
Šumperk 2017	7	10	5	48
Šumperk 2018	27	26	18	0-1 %

Table 2. Monthly temperatures (°C) and precipitation (mm), 2017/2018, ČHMI Opava – Otice

Moon	VIII	IX	X	XI	XII	I	II	III	IV	V	VI
temperature	19.3	13.1	10.5	5.1	2.4	2.4	-3.5	1.2	13.4	16.1	17.6
precipitation	40.2	96.2	56.8	27.3	5.6	27.5	11.1	20.4	4.9	32.9	83.9
Long term normal (1981-2010)											
temperature	18.0	13.4	8.9	3.9	-0.2	-1.3	-0.3	3.4	8.4	13.6	16.5
precipitation	65.4	58.4	33.4	36.0	29.2	18.2	18.6	19.2	40.8	69.3	82.5

Table 3. The influence of fungicidal treatment on health status and yield, variety DK Exlibris, Opava – Chvalíkovice, growing year 2017/2018

Treatment list and timings		Biological activity (%)		Yield (%)
		stalks	roots	
1	Fungicidally and insecticidally untreated control Infestation of pathogen <i>P. lingam</i> on untreated control (intensity %)	0 86	0 91	100 4.06 t/ha
2	Autumn Early spring Flowering Insecticide 1 Insecticide 2 Insecticide 3	6	1	111
3	Autumn Early spring Flowering fungicide 1 - fungicide 3	17	16	107
4	Autumn Early spring Flowering fungicide 1 + insecticide 1 - insecticide 2 fungicide 3 + insecticide 3	18	16	114
5	Autumn Early spring Flowering fungicide 1 + insecticide 1 fungicide 2 + insecticide 2 fungicide 3 + insecticide 3	60	58	133

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**Pathology Session 3:**  
**Recent advances in research of *Sclerotinia*,**  
***Verticillium* and viruses of OSR**



## Forecasting sclerotinia infection in UK oilseed rape

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**Abstract:** *Sclerotinia* stem rot in oilseed rape occurs every year in the UK but incidence varies by region and year, with occasional epidemics that are hard to predict. Control relies on protectant foliar fungicides ahead of any infection, but it is currently difficult to determine the optimum timing of sprays. The aims of this work were to provide forecasting alerts and reports of risk factors for sclerotinia infection to growers during the oilseed rape flowering phase, and demonstrate that the alerts and risk factors helped to improve predictions of sclerotinia disease.

Weather-based alerts were published three times a week for growers (<https://cereals.ahdb.org.uk/>) 2015-2017, for 15 sites/year, based on 48 hr forecast weather data, crop stage and % petals testing positive for sclerotinia. A Burkard trap was deployed at five of these sites to detect airborne spores, and at three of these sites there was a fungicide timing field experiment. Forecast weather data was used to guide fungicide spray timings, for comparison with sprays at yellow-bud, early-, mid- or late-flower. The number of weather based alerts during flowering from 2015-17 varied from zero to three, with the most alerts in the SW and S area. The main difference across the years was the occurrence of alerts in 2017 in E and NE east areas, whereas in 2015 and 2016 these regions were drier and had few alerts.

For analysis of the benefits of risk forecasting, data from fungicide timing experiments in 2015-2017 was combined with data from similar experiments in 2010-2012, to give a total of 23 site-years. Zero weather-based alerts were a correct predictor of no- or low-infection ( $\leq 1\%$  stem rot incidence). But where there were weather-based alerts (at 15 sites), about half of these sites (7) also had an infection incidence of  $\leq 1\%$ . Including the results of sclerotinia inoculum on petals with analysis of weather alerts helped to improve the predictions of infection, by correctly identifying the very-low risk sites. The relationship between % of petals testing positive and untreated disease incidence varied with timing of sample (e. g.  $R^2 = 0.54$  for petals sampled at yellow-bud), but when combined with weather-based alerts, petal data improved the accuracy of the risk forecast. In summary, when inoculum was zero, infection risk was zero. Positive inoculum indicated risk, but infection was variable. Spore trap results helped explain where infection did or did not occur, by showing the variation during flowering of daily levels of spores in air samples for each site.

**Key words:** *Sclerotinia*, oilseed rape, disease forecasting, integrated control

### Introduction

*Sclerotinia* stem rot in oilseed rape occurs every year in the UK, at generally low levels but with occasional major outbreaks which are difficult to predict. Infection occurs mainly during the flowering phase, via petals (Young & Werner, 2012). The long-term mean UK sclerotinia incidence from 2006-2015 was 14% crops and 2% stems affected, based on fungicide treated



crops ([www.CropMonitor](http://www.CropMonitor)). In the past two years, incidence has declined to 8% crops and 1% stems affected in 2016 and 2017, respectively. But the use of fungicides targeted at sclerotinia has remained at similar levels each year since 2011, with 95% or more of the UK oilseed crop area treated three times with fungicides (Pesticide Usage Statistics, <https://secure.fera.defra.gov.uk/pusstats>), and with over 50% of fungicide applications timed during flowering (Garthwaite *et al.*, 2016) and targeted mainly at sclerotinia control. However, high disease pressure has occurred in recent years in some regions, as seen in untreated trial plots, e. g. 43% incidence in 2017 (Gosling & Ritchie, 2018). Therefore, infection risk can be high and the use of protectant fungicides is justified in some cases, but not all. The aims of this work were to analyse the use of weather-based forecasting alerts and infection-risk factors for sclerotinia, to provide improved guidance to growers as to the need for fungicide treatment, and if needed, the optimum time to apply.

## Material and methods

Sclerotinia infection risk factors were recorded during flowering from sites across England and Scotland in 2015-17, as follows. For 15 sites in total per year, 48 hr forecast hourly weather data (air temperature, relative humidity and rain) was purchased from the UK MetOffice ([www.metoffice.gov.uk](http://www.metoffice.gov.uk)), and crop growth stage and % petals testing positive for sclerotinia (agar plate test) were recorded at intervals during flowering. At three of the 15 sites, field experiments were set up to test control from fungicides applied once, timed according to weather-based alerts, or growth stage: yellow-bud, early-flower, mid-flower or late-flower. At five of the 15 sites, air-sampler traps to detect airborne spores were deployed.

The forecast weather data was used to calculate if and when infection alerts were predicted. Results were updated three times a week by e-mail to each site during flowering. Alerts were based on conditions conducive to infection by sclerotinia, which were: temperatures  $\geq 7$  °C and RH  $\geq 80\%$  for  $\geq 23$  consecutive hours (based on Koch *et al.*, 2007). Burkard spore traps, located within each field trial, were run continuously from prior to flowering to after end of flower. The Burkard drums were changed weekly and tested by qPCR (Rogers *et al.*, 2009), to give daily amounts of sclerotinia DNA. Petals were sampled just before each fungicide timing, and tested on agar plates (Young *et al.*, 2014). For analysis of the benefits of risk forecasting, data from fungicide timing experiments in 2015-2017 was combined with data from similar experiments in 2010-2012, to give a total of 23 site-years. Sites were allocated into categories of stem incidence  $> 1\%$ , or  $\leq 1\%$ , and these categories were subdivided into the number of sites with both a weather-based alert and positive petals, or sites which experienced neither of these factors, or one factor only.

## Results and discussion

### *Pattern of weather based infection alerts*

The number of forecast weather based alerts during flowering from 2015-17 varied from zero to three at individual sites (Figure 1), but with many more occurring outside of flowering, mostly afterwards with increasing temperatures, particularly at night time. In practice, this means there are relatively few occasions during flowering where weather conditions are conducive to infection, and depending in the year, a fungicide application could be timed in response to the first infection alert during flowering. In the UK, flowering onset and duration can vary widely within regions, and between fields and varieties, and it is important to

monitor crop flowering progress locally to help with assessing sclerotinia infection risk. In 2015 and 2016, the alerts occurred in the south and south west regions, which is the most commonly seen distribution, with eastern crops generally assumed to be at less risk. However, in 2017 there were more alerts in the east and north east regions, where the limiting factor is usually lower humidity, but in these years there was higher than normal eastern rainfall and coastal mists and consequent higher humidity.

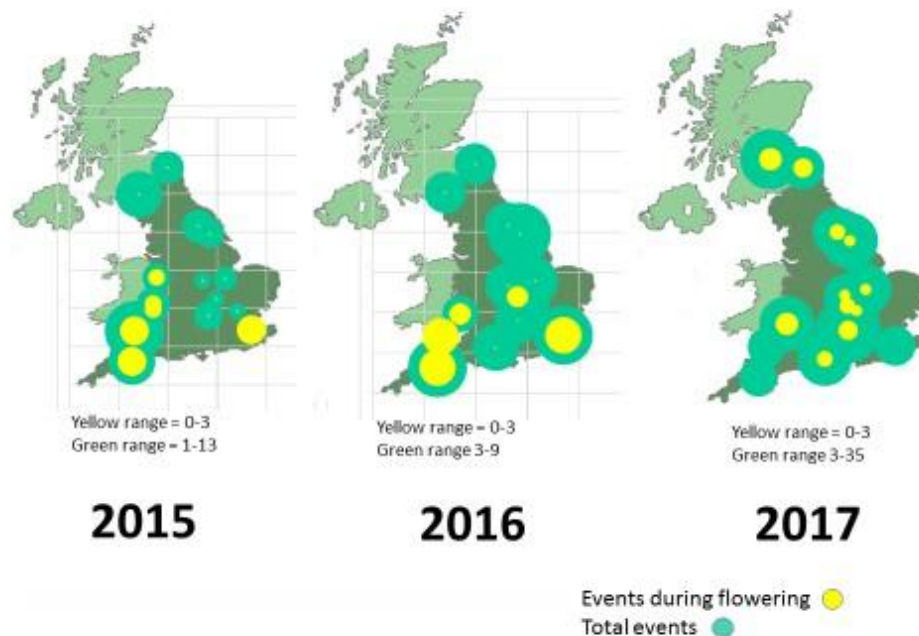


Figure 1. Number of weather-based sclerotinia infection alerts in England and Scotland during oilseed rape flowering (inner circles) and cropping season (outer circles), 2015-17.

### *Sclerotinia inoculum*

Spore trap results helped explain where infection did or did not occur, by showing details of daily levels of spores in air samples varying during flowering for each site. For example, in 2016 (Figure 2) there were peaks of airborne spore numbers during mid-flower for the Devon site in the south west, and during late-flower in particular for the Herefordshire site in the west midlands. The timing of spore peaks coincided with some of the weather-based alert occurrences and helped explain final disease incidence. For example, at the Herefordshire site in 2016 there were spore peaks of 380 spores/m<sup>3</sup> of air on 19 April during the early-mid flower growth stage (Figure 2), a key infection risk phase, which led to 5% incidence on main stems in untreated plots. The Herefordshire site also had a high late-flower peak of over 1400 ascospores per m<sup>3</sup> of air on 8<sup>th</sup> May 2016 (Figure 2) which very likely explained the incidence of 3% Sclerotinia stem rot on lateral branches in untreated plots at the same site. By contrast, there were low levels of spores during flowering at the Lincolnshire site in the east. There was high spore production after flowering had ended at the Lincolnshire site (Figure 2, 2784 spores/m<sup>3</sup> of air, but this did not pose an infection risk, as evidenced by lack of sclerotinia disease development in the crop.

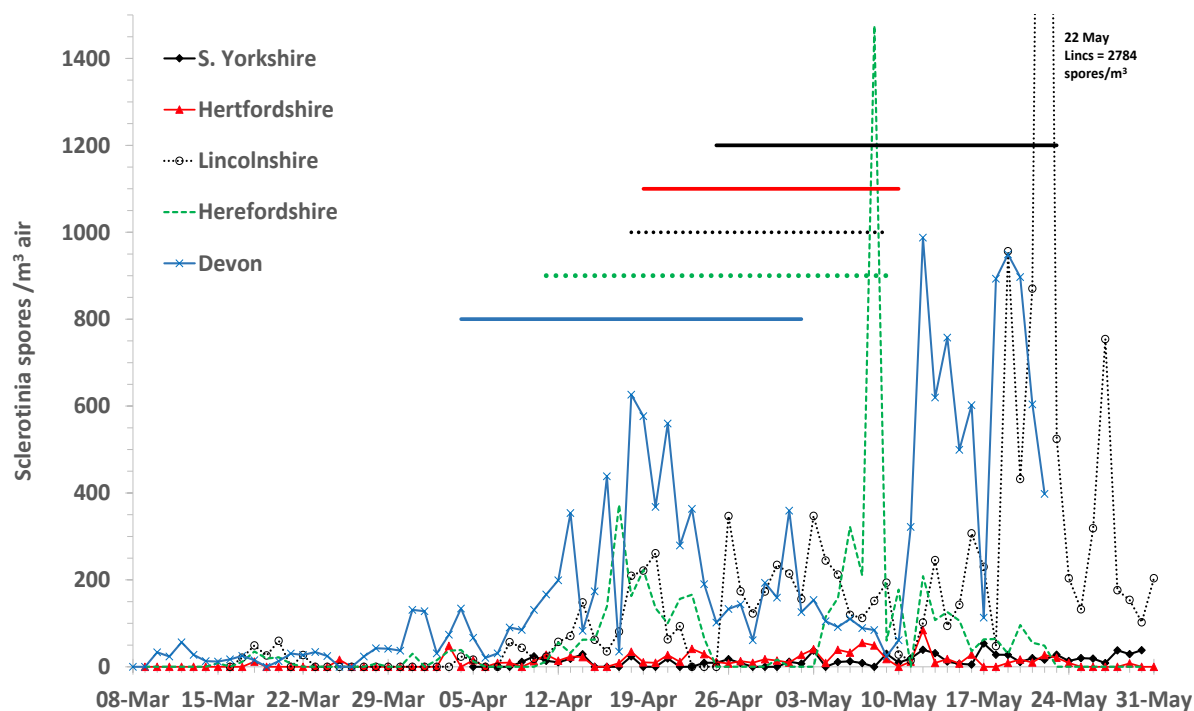


Figure 2. *Sclerotinia* ascospore inoculum in UK oilseed rape field trials 2017.

The relationship between % of petals testing positive and untreated disease incidence varied with timing of sample, with the best results seen from the earliest sample time at yellow-bud ( $R^2 = 0.54$ , Figure 3). Petal testing has been used to help evaluate *sclerotinia* infection risk with some promising results (e. g. Turkington *et al.*, 1991; Becca *et al.*, 2016) and developments with molecular tests have speeded up test-result availability such that results could be obtained in time to make spray decisions (e. g. Ziesman *et al.*, 2016). However, petal results alone, even if tests are quick, are unlikely to be good predictors of infection levels unless interpreted alongside weather conditions. The results from agar plate tests in the current work show that zero-petal infection is a good predictor of very low infection. However, if petals test positive, the relationship between % petals positive and final stem rot incidence is variable. A quicker test for *sclerotinia* in-field would be useful (e. g. Almquist *et al.*, 2015), but further work is needed to develop a cost-effective test, ideally for use in-field.

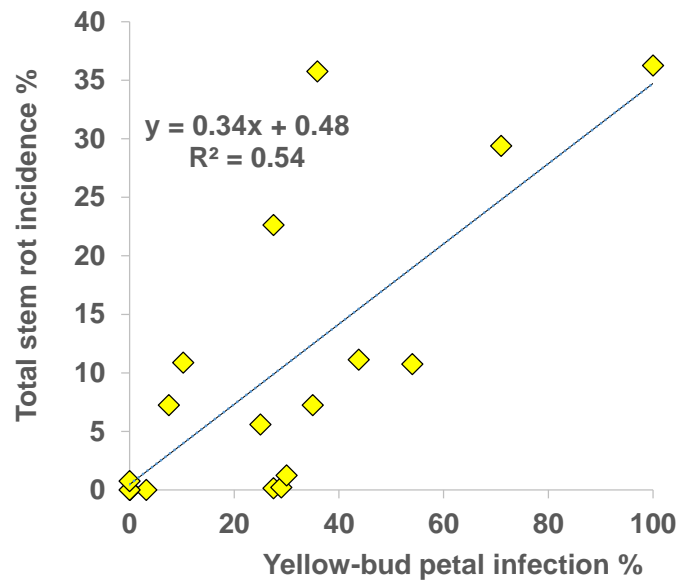


Figure 3. Percentage of oilseed rape petals sampled at yellow-bud stage testing positive for sclerotinia, and % incidence stem rot pre-harvest, UK field experiments 2010-2017.

#### ***Evaluation of predictions, based on weather and inoculum***

Allocating sites to categories according to sclerotinia infection and risk factors showed the number of cases of false negatives and true positives. It is particularly important for disease forecasting schemes to minimise false negative predictions, i. e. where disease is not forecast and no treatment is advised, but disease occurs. It is also important to minimise the occurrence of situations with false positives, where the forecast indicates high risk, and treatment is applied but in hindsight was not justified. In the current work, minimising the occurrence of false negative situations was achieved by setting a requirement for sites to experience both a weather based alert and a positive petal test result with a threshold of  $\geq 5\%$  with sclerotinia. Sites experiencing both factors were correctly predicted to be at risk, i. e. they had subsequent disease (Table 1; 13 sites with sclerotinia stem rot  $> 1\%$ ). Sites experiencing only one of the factors, or neither, had zero stem rot (Table 1; zero sites with sclerotinia stem rot  $> 1\%$ ) or very low stem rot (Table 1; 10 sites with stem rot  $\leq 1\%$ ). By contrast, there were no false positives using these criteria (Table 1: zero sites with sclerotinia stem rot  $\leq 1\%$ ). Therefore, combining the results of agar plate petal tests with the occurrence of weather-based alerts helped to improve the predictions of infection, by correctly identifying the very-low risk sites which would in hindsight not normally justify a fungicide treatment.

Table 1. Evaluation of predictions of sclerotinia stem rot occurrence in UK oilseed rape field experiments 2010-2017 (23 sites).

	NUMBER OF SITES	
	Sclerotinia stem rot incidence > 1%	Sclerotinia stem rot incidence ≤ 1%
<b>Weather alert* AND ≥ 5% petals** positive</b>	<b>13</b>	<b>0</b>
<b>Weather alert OR petals positive OR neither</b>	<b>0</b>	<b>10</b>

\*Weather alert based on infection criteria in Koch *et al.*, 2007.

\*\*40 petals tested by agar plating; positive result threshold = ≥ 5% with sclerotinia.

In summary, (a) when there were no weather based alerts, or inoculum was zero, infection risk was zero, and (b) the occurrence of weather-based alerts and positive inoculum correctly predicted infection, but the infection levels were variable. Spore trap results helped explain where infection did or did not occur, by showing daily levels of spores in air samples varying during flowering for each site. Sclerotinia forecasting schemes based on weather, crop stage and agronomic factors, but not including inoculum measurements, can be reliable if it is established that inoculum is always present and therefore not limiting (Koch *et al.*, 2007). The current study has shown that there is great variability in the presence of sclerotinia inoculum in the UK, which most likely explains why inclusion of inoculum detection in weather-based infection models helps to improve the accuracy of sclerotinia infection predictions. The challenge now is to improve the forecasting to discriminate between sites which have high infection, and those which have low infection which will not justify a fungicide treatment.

## Acknowledgements

We thank AHDB for funding the field trial and spore detection work, BASF for funding field trials, and the excellent ADAS field team staff involved in the field operations from 2010-2017.

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## Detection of ascospore release of *Sclerotinia sclerotiorum* with real time PCR: an important tool in understanding disease development in winter OSR

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**Abstract:** *Sclerotinia* stem rot, caused by *Sclerotinia sclerotiorum*, has turned into a major disease of winter oilseed rape (OSR) in Sweden. Detecting DNA from leaf samples and from daily air samples along with field assessments has given an increased knowledge of disease development.

**Key words:** *Sclerotinia sclerotiorum*, qPCR, stem rot

### Introduction

*Sclerotinia* stem rot, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, has been a major disease of spring oilseed rape (OSR) in Sweden. In recent years severe outbreaks were frequently reported in winter OSR south and south central Sweden. The impact of *Sclerotinia* stem rot is dependent on weather conditions and the timing of ascospore release. Injuries caused by *Sclerotinia* stem rot was the individually most important cause of yield penalty in 2017, following a season of conducive weather conditions.

The disease forecasting service available to Swedish farmers is a regional risk assessment based on local climate and field information. A review of a Canadian system for creating disease risk maps based on data such as precipitation, temperature and soil moisture showed that the disease forecast correlated well with actual disease development on a regional scale, but was not satisfactory for individual fields (McLaren *et al.*, 2004).

A real-time PCR assay was developed and used to determine the incidence of *S. sclerotiorum*. DNA on petals and leaves of spring OSR as well as in air samples of a Burkhard spore sampler, with the aim of finding tools to improve precision in disease risk assessment (Almquist & Wallenhammar, 2014). The objectives of this study were to increase our knowledge to understand disease development in individual fields of winter OSR by using quantitative detection of *S. sclerotiorum* on leaves of winter OSR and in air samples, and by determining the occurrence of infected plants in a region in south central Sweden where winter OSR previously rarely was grown.

## Material and methods

### *Selection of field sampling areas*

Ten fields were selected each year in collaboration with the extension staff at the Rural Economy and Agricultural Society, Örebro. Early in the month of May at budding stage sampling areas covering the width of the sprayer, most commonly 24 meters × 100 m were selected and marked with specially designed sticks. The farmers left the sampling area unsprayed if fungicides were to be applied in the field.

### *Field sampling of leaves and air sampling*

Field samples were collected in fields of winter OSR during three seasons from 2015 to 2017. The leaves were collected at BBCH 63. From ten randomly selected plants the bottom leaves were detached. The detached leaves were placed individually in plastic zip lock bags and stored in a freezer until DNA extraction. Air samples were collected by a Burkard 7-day continuously recording spore sampler placed within the sampling area in one of the fields. Air was sampled continuously from early May to early July. Daily samples were prepared according to Almquist & Wallenhammar, 2015.

### *Sclerotinia stem rot assessment*

Disease incidence was determined using 100 plants at development stage BBCH 80 collected randomly in the sampling area in early July. Disease severity was assessed and a disease severity index calculated.

### *DNA extraction and qPCR analyses*

DNA extraction and qPCR analyses were performed according to Almquist & Wallenhammar, 2015.

## Results and discussion

In 2015 and 2016 the incidence of stem rot was equal to or above 15% in five out of ten and nine out of ten winter OSR fields, respectively. The highest infection level, 62%, was assessed in a field in 2016. Leaves from OSR are still being analyzed. The results from the spore trap showed that *S. sclerotiorum* DNA was detected in the air from the beginning of May until the beginning of July. The obtained qPCR data in combination with stem rot incidence and climate data are being employed in the development of a computational prediction model that could potentially be used to improve disease risk assessment. Preliminary results will be discussed. The results are of great importance for Swedish winter OSR growers as < 90% of the OSR acreage constitutes of winter OSR.

## Acknowledgements

We thank the KK foundation and the Swedish Farmers Research Foundation for funding and Martin Pettersson, Rural Economy and Agricultural Society, Örebro, for collaboration finding OSR fields.



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## Efficacy evaluation of different group of fungicides on control of *Sclerotinia sclerotiorum* and yield of winter oilseed rape

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**Abstract:** *Sclerotinia* stem rot (SSR), caused by *Sclerotinia sclerotiorum*, is a major disease of oilseed rape worldwide. Leaves, stems and pods at different developmental phases can be infected. Under more intensive cultural practices, fungicides have been applied to control SSR (Bardin & Huang, 2001). Till date, few studies were conducted to compare the effectiveness of biological control agents with fungicides. During current study, the pathogen was assayed for sensitivity to different groups of fungicides alone or in combinations (SDHI, QoI, DMI, Inhibitor of MAP) and a biological compound (*Bacillus subtilis* strain QST 713) in pure cultures on agar medium and in experimental field plots. The field trial was conducted in Salzdahlum, Lower Saxony, Germany in 2016/2017 to evaluate the effect of fungicides on *Sclerotinia* stem rot control, yield and quality parameters of the seed. To have a homogeneous *Sclerotinia*-infection inside the field, OSR plants were artificially inoculated with mycelia-oat inoculum (50 g per m<sup>2</sup>) at mid of flowering stage (BBCH-Scale 64-65). Directly after inoculation, 10 mm irrigation water was applied equally to all plots every other day until 10 dpi. One day after inoculation, fungicides were applied to the plants at the manufacturer's recommended dose rate. The treatments were arranged in a randomized block design with four replications. The severity of *Sclerotinia* stem rot was assessed once at growth stage (BBCH) 81-83 on 100 randomly plants from each plot. The plots were harvested at the end of season and seed yield was determined per hectare. Oil and protein content as well as the amount of glucosinolates were measured by using near infrared spectroscopy. In vitro sensitivity evaluation of *S. sclerotiorum* isolates to fungicides carried out by analyzing the distribution of EC<sub>50</sub> values and percent of growth inhibition of 30 isolates. Sensitivity tests were conducted using fungicide amended potato dextrose agar plates at 0.0, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0 µg a.s./ml concentrations.

The results from field trial showed that the occurrence of SSR is strongly depending on presence of the inoculum and plant wetness. Significant differences in disease severity were observed between artificial inoculated plots and non-inoculated ones. Disease severity index in non-inoculated plots was 4.2%, while in inoculated controls it was up to 82%. All fungicide treatments as well as the biological agent significantly reduced the disease severity (36-87%) and glucosinolate content (14-25%) and increased yield (55-99%), TGW (1.5-9.8%), and oil content (1.4-5.5%) as compared with the untreated control. Among all treatments, applications of fludioxonil and boscalid + pyraclostrobin combination have the best effect on the reduction of disease and yield loss.

In vitro study showed that mycelial growth was completely prevented by all fungicides. However, metconazole, tebuconazole and prochloraz were significantly more effective compared to azoxystrobin and triadimenol by showing greater antifungal activity in lower concentrations. Furthermore, the biological agent had a broad antagonistic spectrum and significantly reduced the mycelial growth of *S. sclerotiorum* isolates and suppressing sclerotia formation. EC<sub>50</sub> values for metconazole and prochloraz were the lowest of the fungicides

used. Within species the highest variability of EC50 values was observed towards azoxystrobin.

The results of this study strongly suggest that using *Bacillus subtilis*, as a biological agent, is a promising method for control of *Sclerotinia* stem rot in oilseed rape cultivation.

**Key words:** *Brassica napus*, *Sclerotinia* stem rot, SDHI, QoI, DMI, biological control, *Bacillus subtilis*

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## Evolution of SDHI fungicide resistance in *Sclerotinia sclerotiorum* in France

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**Extended abstract:** Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* (Lib) de Bary is a major disease on winter oilseed rape in France. Due to the lack of resistant varieties, the control of SSR is mainly based on the application of preventive fungicides, sometimes with biological control agents. Five fungicide classes with various modes of action are effective against SSR: benzimidazole anti-microtubules, dicarboximides affecting osmoregulation, demethylation inhibitors (DMIs), inhibitors of complex III of respiration (QoIs) and inhibitors of complex II of respiration (SDHIs). Only the last three modes of action remain registered in France and the evolution of resistance is monitored yearly.

The samples were collected by a partnership between Terres Inovia and French official Plant Protection Services (DGAL-SDQPV). They represented all the French rapeseed regions. Sclerotia were collected from symptomatic rapeseed stems, one sclerotia per plant and one dozen sclerotia per field. Then the sclerotia were analyzed by Anses and Terres Inovia with the scientific and methodological support of INRA. The phenotypic test, used for detecting boscalid resistant isolates, is based on the measurement of mycelial growth on minimal agar medium, with succinate as carbon source. Medium was amended with the discriminatory dose of boscalid 2 mg/l.

A HRM (High Resolution Melt) method was also developed by Terres Inovia to detect the main mutations potentially involved in resistance. Preliminary sequencing of resistant strains located more than 10 mutations in the genes encoding the subunits B, C or D of the succinate dehydrogenase, the target of SDHIs. Four pairs of HRM primers (one for SdhB and SdhC and two for SdhD) were developed and used in routine to characterize the genotype of the resistant strains.

The HRM analyses revealed that genotypes D-H132R and C-H146R represented 80% of the resistant genotypes isolated since 2011. These strains are highly resistant to boscalid (subgroup of pyridines) and weakly to moderately cross-resistant to pyrazoles SDHIs (e. g. bixafen, fluxapyroxad) and to fluopyram (subgroup of benzamides).

The first strains of *S. sclerotiorum* resistant to SDHIs were detected in 2008 after two seasons of commercial use of boscalid. Then, their detection has slowly increased to reach 2% of sampled strains in 2012. In 2014, the resistance has exponentially expanded, and was detected in 30% of sampled locations and in more than 15% of the analyzed sclerotia. Despite a low disease pressure over the past three years, this resistance was present in 40-50% of the locations (Figure 1). The increasing proportion of resistant sclerotia in samples from the same period has to be interpreted with caution, considering the limited number of samples, related to low disease pressure.

Because knowing the sensitivity of the strains of all the fields is impossible, the management of this mode of action is without distinction all the active ingredients of the SDHI group.

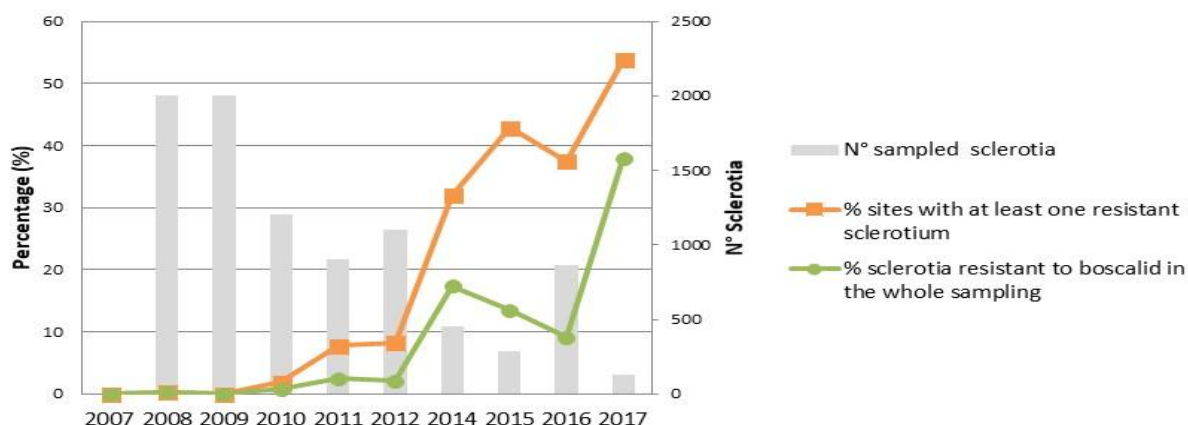


Figure 1: Temporal evolution of boscalid resistance in *S. sclerotiorum* in France

Monitoring results, as well as recommendations on SDHI resistance management, are communicated to farmers yearly. They promote an integrated approach for controlling SSR and, hence, to implement:

- 1) Farming practices for reducing the soil-born sclerotia by
  - ✓ Extending the rotation of OSR with less susceptible crops
  - ✓ Using biological control with *Coniothyrium minitans* to reduce soil infestation and thus the risk of sclerotinia in following years.
- 2) Strategies for managing fungicide resistance:
  - ✓ Only use fungicide when necessary, after considering information from crop monitoring and disease forecasts;
  - ✓ When a protection is required,
    - Avoid the use of SDHI fungicide alone,
    - Use only one application of SDHI fungicide per season on rapeseed.
  - ✓ In situation of resistance to SDHIs, with a high risk of sclerotinia disease,
    - Use different modes of action on rapeseed,
    - Achieve a SDHI break on rapeseed, at least one year on each plot, by using other modes of action,
  - ✓ avoid mixtures of SDHI + QoI, as well as specialties whose efficacy is based mainly on QoI compound efficacy. This is to ensure the sustainability of this mode of action. Prothioconazole-based solutions, or mixtures with triazoles are probably the most sustainable strategies. Moreover, mixtures of half-dose fungicide with a biocontrol agent are not suitable for managing resistance due to the limited efficacy of biological agents.

Strategies based on 2 fungicide applications (with an interval of 10 to 15 days) do not improve SSR control and increase the resistance risk. They are rarely valuable, except for control of other diseases such dark spot, ring spot or a high pressure of powdery mildew.

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## Biocontrol potential by the apathogenic A1/D2 lineage of *V. longisporum* against pathogenic strains of *V. longisporum* and their interaction with roots of oilseed rape

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**Abstract:** *Verticillium longisporum* is an amphidiploid fungal pathogen found in three different lineages putatively derived from three independent hybridization events. The lineage A1/D2 has only been found in horseradish in the USA, it mostly exhibits low level aggressiveness in its host and is apathogenic in most *Brassica* crops. Apathogenic strains of plant pathogens have proven in the past to be candidates for biological control of aggressive strains of that particular fungal species. Thus, the biocontrol potential of this *V. longisporum* lineage was tested with root dip-inoculation of oilseed rape (*B. napus*) seedlings, where A1/D2 isolates were separately inoculated a week prior or simultaneously to the inoculation with an aggressive isolate of *V. longisporum*. A reduction of symptoms was observed for both types of inoculation with A1/D2. Observations with confocal microscopy of the A1/D2 interaction with roots of oilseed rape seedlings suggest that its biocontrol effect might be due to a competition for space at the root surface level, indicated by ample sporulation and mycelial growth on the root.

**Key words:** *Verticillium longisporum*, lineages, biological control, *Brassica napus*, oilseed rape

### Introduction

*Verticillium longisporum* is an amphidiploid fungal pathogen that consists of three different lineages putatively originated from three independent hybridization events from at least two different lineages of *V. dahliae* (D2 and D3) and two unknown species (A1 and D1). Thus, the three lineages of *V. longisporum* are referred to as A1/D1, A1/D2 and A1/D3. The lineage A1/D2 has only been found in horseradish in the USA, it mostly exhibits low aggressiveness in its host and is apathogenic in most *Brassica* crops (Inderbitzin *et al.*, 2011; Novakazi *et al.*, 2015). Apathogenic strains are good candidates for biological control of a fungal pathogen (Deketelaere *et al.*, 2017). Thus, the potential of *V. longisporum* A1/D2 lineage as a biocontrol agent in oilseed rape against the most frequent and aggressive lineage in oilseed rape (A1/D1) is investigated in this study. Finally, in an attempt to try to elucidate if a biocontrol effect is due to competition for space, the colonization patterns of the A1/D2 at the root surface was microscopically investigated.

## Material and methods

### *Study of biocontrol potential of V. longisporum A1/D2 against aggressive V. longisporum isolates in oilseed rape*

The biocontrol potential of this *V. longisporum* lineage was tested with root dip inoculation of 2 weeks-old oilseed rape seedlings, where spores ( $10^6$  spores/ml) of three A1/D2 isolates were separately inoculated a week prior or simultaneously to an aggressive A1/D1 isolate of *V. longisporum*. To assess symptom development, AUDPC and stunting were calculated.

### *Microscopical studies of the interaction of A1/D2 at the root surface of B. napus*

To assess the interaction of A1/D2 with roots of oilseed rape, seedlings of oilseed rape were grown *in vitro* on PNM medium and roots were spray-inoculated with a spore suspension of A1/D2 ( $10^6$  spores/ml). Microscopic examinations took place at 1, 3 and 7 dpi. A double staining with Alexa Fluor (WGA-AF 448) (50  $\mu$ g/ml) and propidium iodide (10  $\mu$ g/ml) was carried out for confocal microscopy.

## Results and discussion

Preliminary experiments show a lineage-specific biocontrol effect of A1/D2 against the aggressive A1/D1 isolate of *V. longisporum* with both inoculation methods. The experiment will be repeated with other A1/D1 isolates.

Preliminary observations with confocal microscopy of the A1/D2 interaction on roots of oilseed rape seedlings suggest that its biocontrol effect might be due to a competition for space at the root surface level, indicated by ample sporulation and mycelial growth on the roots at 3 dpi.

## Acknowledgements

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## Lineage monitoring of *V. longisporum* in European and Canadian oilseed rape fields and first steps towards analysis of genetic diversity using GBS

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**Abstract:** *Verticillium longisporum* is an amphidiploid fungal pathogen specialized on Brassicaceae hosts of which three genetically different lineages have been found so far in the field. Previous studies have shown that the lineages of *V. longisporum* differ in their frequency, occurrence, distribution, and host preferences. To further investigate the distribution of lineages in European oilseed rape fields, we carried out, for the first time, a large scale *V. longisporum* lineage monitoring with stubble samples collected during four years (2013-2016) from 52 locations in seven European countries, which resulted in a total of 306 isolates. Using a Multiplex-PCR, all isolates were assigned to the lineage A1/D1. The broad temporal and geographic scope of this monitoring demonstrates a dominant role of lineage A1/D1 in European oilseed rape fields. To further investigate whether genetic subpopulations exist within the lineage A1/D1 with different pathogenic traits, a selection of 96 isolates will be genetically characterized with genotyping by sequencing (GBS) for a further in-depth study of genetic diversity.

**Key words:** *Verticillium longisporum* lineages, oilseed rape, GBS, *B. napus*

### Introduction

*Verticillium longisporum* is an amphidiploid fungal pathogen found in three different lineages putatively originated from three independent hybridization events from at least two different lineages of *V. dahliae* (D2 and D3) and two unknown species (A1 and D1). Thus, the three lineages are referred to as *V. longisporum* lineage A1/D1, A1/D2 and A1/D3 and present different geographic distribution and host preference of Brassica crops (Inderbitzin *et al.*, 2011; Inderbitzin *et al.*, 2013). The objective of this study was to carry out the first large-scale *V. longisporum* lineage monitoring in European fields. A previous study has shown that the *V. longisporum* A1/D1 lineage consists of two genetically distinct groups (Depotter *et al.*, 2017). Therefore, in order to further investigate the diversity of this lineage, a GBS analysis of a high number of A1/D1 isolates will be used to increase the resolution of the phylogenetic relationships.

## Material and methods

### *V. longisporum* lineage monitoring

Through a cooperation network, stubbles from oilseed rape fields were obtained. For isolation, epidermis fragments (3 mm) of infected stubbles were disinfected and plated on SNA medium. DNA from single spore cultures was used to carry out a multiplex PCR assay (according to Inderbitzin *et al.*, 2013) to characterize the three lineages of *V. longisporum*.

### *Isolate selection and DNA extraction for GBS analysis*

For the GBS analysis, 96 isolates of *V. longisporum* were selected, from which 82 are A1/D1 European isolates. The selection of isolates was based on geographic reasons and ensuring that at least three isolates of the same location are available. Additionally, reference isolates from the three lineages, as well as Canadian isolates – of which the lineage has not yet been characterized – will also be used in the analysis. For the DNA extraction, a modified protocol according to Xin & Chen (2012) is carried out to obtain high quality DNA with a low carbohydrate content.

## Results and discussion

### *V. longisporum* monitoring

In total, 302 isolates were obtained from stubbles collected between 2013 and 2016 from 52 locations in 7 countries. With the multiplex-PCR, all isolates were categorized as lineage A1/D1. Therefore, it can be concluded that the lineage A1/D1 is the dominant lineage in European oilseed rape fields and that a more detailed differentiation of the lineage A1/D1 is needed.

### *Isolate selection and DNA extraction for GBS analysis*

The modified DNA extraction protocol produces high yields of DNA – up to 250 ng/μl – , which are suitable for the GBS analysis. It is expected to detect genetically distinct A1/D1 populations with the phylogenetic analysis from data generated by GBS. These results are intended to be used to determine if different genetic populations represent different pathotypes.

## Acknowledgements

We thank Dagmar Tacke, Isabel Müller and Isabella Reitz for their technical assistance.

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## **Virus diseases of oilseed rape (*Brassica napus* subs. *napus*) in the Czech Republic**

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**Abstract:** In autumn 2016 the unusually high abundance of green peach aphid (*Myzus persicae*) occurred on oilseed rape fields across the Czech Republic. This species is a vector of *Turnip yellows virus* (TuYV) and *Turnip mosaic virus* (TuMV), which are commonly found on oilseed rape. The nationwide monitoring of these two viruses was made using detection by Triple Antibody Sandwich Enzyme-Linked ImmunoSorbent Assay (TAS-ELISA) and Double Antibody Sandwich ELISA (DAS-ELISA). The test revealed high occurrence of TuYV – 93.7% of tested samples were positive. On the other hand, the occurrence of TuMV was very low – just 0.2% of samples were positive. In spring 2017 the monitoring were targeted on TuYV occurrence in oilseed rape cultivars, one of them resistant. Totally, 3221 samples were tested. Only 31 (0.96%) samples were negative, the rest – 3195 (99.04%) were positive on TuYV. Very low correlation between cultivar resistance and level of absorbance measured in ELISA test was proven. There were low differences in yield between resistant and susceptible cultivars. In spring 2018, another testing of resistant susceptible cultivars was realized. Totally 512 samples were tested, the result will be analysed.

**Key words:** *Myzus persicae*, *Turnip yellow virus* (TuYV), *Turnip mosaic virus* (TuMV), ELISA, absorbance, resistant cultivars

### **Introduction**

*Turnip yellows virus* is the most important viral disease, which is occurring on oilseed rape in many places in the world (Stevens *et al.*, 2008). There is no available direct chemical control against this pathogen. Methods of control consist of breeding brand new varieties of plants with TuYV resistance or also genetically modified plants or of direct control against the main vectors of infection like green aphid.

*Turnip yellows virus* causes variable disease symptoms on plants from different families, with the most important of these hosts are Brassicaceae (Graichen & Rabenstein, 1996). Major symptoms include yellowing of leaf veins and reddish leaf edges (Stevens *et al.*, 2008). Various aphid families are responsible for the transmission of this virus, but the main vector is *Myzus persicae* (Schliephake *et al.*, 2000). TuYV infection of oilseed rape crop leads to production and quality losses ranging from 9-46% and reduced fatty acid content and increased content of indole glucosinolates (Coleman, 2013).

## Material and methods

### *Samples*

Totally 503 samples for testing in autumn 2016 were acquired from 58 localities across the Czech Republic, 3221 samples tested in spring 2017 were obtained from 40 localities in the Czech Republic and Slovakia. Totally 341 samples came from common fields and 2280 samples from cultivar trials (CT) held by Union of Oilseed Growers and Processor or from trials with recommended cultivars (TRC) held by Central Institute for Supervising and Testing in Agriculture. The resistant cultivar was not present in all trials.

### *Serological testing*

Testing was done by Triple Antibody Sandwich Enzyme-Linked ImmunoSorbent Assay (TAS-ELISA) and Double Antibody Sandwich ELISA (DAS-ELISA). The antibodies were obtained from Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

## Results and discussion

Totally, 99.04% samples were positive on TuYV. The one resistant cultivar had 94.6% positive samples on 10 localities. In cultivar trials, the average yield of resistant cultivars did not correlated with absorbance measured in ELISA test and it was similar to susceptible cultivars, which is shown in Figure 1. In trials with recommended cultivars had the resistant cultivar higher yield (120% on control) than other susceptible cultivars and level of absorbance was lower, shown in Figure 2.

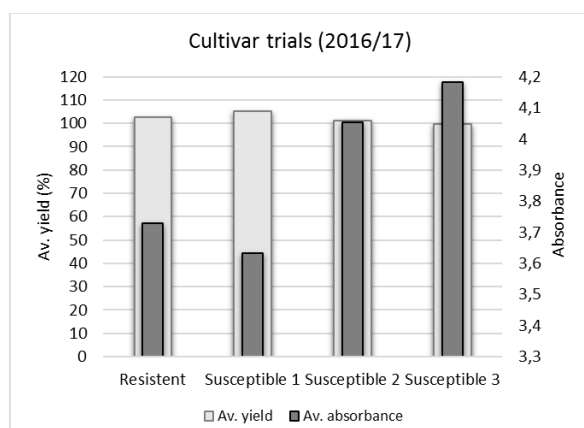


Figure 1. Average yield and level of absorbance measured in ELISA test for cultivar trial in 2016/17.

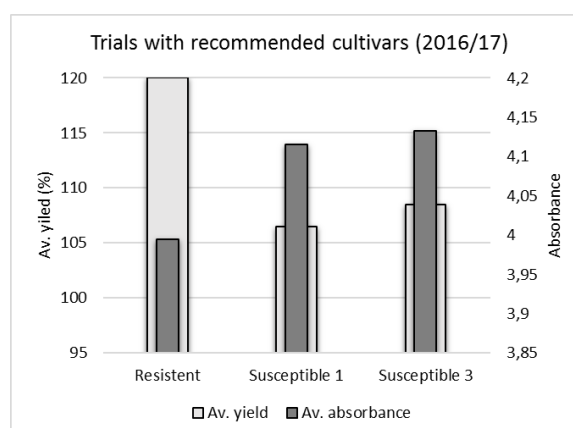


Figure 2. Average yield and level of absorbance measured in ELISA test in trials with recommended cultivars 2016/17.

The season 2016/17 brought heavy TuYV infection of oilseed rape and very small differences between cultivars were observed. The testing made in season 2017/18 is promising and some differences were already visible during ELISA testing.

## Acknowledgement

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