



IOBC-WPRS

IOBC –WPRS MEETING

Working Group “Integrated Control in Oilseed Crops”

Convenor: Małgorzata Jędrzycka

SG Entomology Convenor: Sam Cook

SG Pathology Convenor: Małgorzata Jędrzycka

**On line meeting
May 17th-18th, 2022**

Organizers:

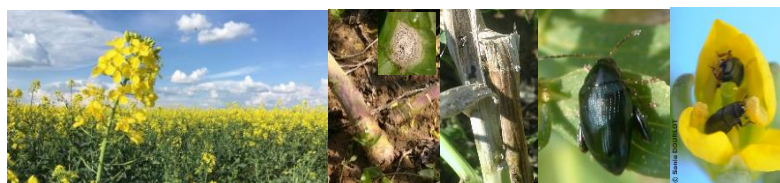
Anne-Marie Cortesero

Régine Delourme

Maxime Hervé

Nathalie Quéré

Institute for Genetics, Environment and Plant Protection



IOBC-ICOC meeting - Schedule

1st day – 17th May (TUESDAY)

- 09:00 – 09:15 Opening of the Meeting
- 09:15 – 09:30 Introduction on IOBC
- 09:30 – 10:30 **PLENARY talks (joint session)**
- 10:30 – 10:45 Coffee Break

PATHOLOGY Sessions

- 10:45 – 12:10 **Pathology session 1:** Blackleg, update on *L. maculans* populations
- 12:10 – 13:10 Lunch Break
- 13:10 – 14:10 **Pathology session 2:** Blackleg Resistance
- 14:10 – 14:30 Coffee Break
- 14:30 – 15:10 **Pathology Session 3:** Diversity of blackleg fungi & associated mycobiota
- 15:10 – 15:30 Overall discussion on Blackleg

ENTOMOLOGY Sessions

- 10:45 – 12:10 **Entomology session 1:** Insecticides: pyrethroid resistance and alternatives
- 12:10 – 13:10 Lunch Break
- 13:10 – 14:50 **Entomology session 2:** Breeding OSR for resistance to insect pests & identification of traits
- 14:50 – 15:10 Coffee Break
- 15:10 – 17:10 **Entomology Session 3:** Behaviour & ecology of OSR pests: towards IPM
- 17:10 – 17:30 Discussion from Day 1

IOBC-ICOC meeting - Schedule

2nd day – 18th May (WEDNESDAY)

09:15 – 10:15 **POSTER session - flash talks separate sessions** Entomology/Pathology

10:15 – 10:30 Coffee Break

PATHOLOGY Sessions

10:30 – 12:30 **Pathology session 4:** OSR diseases and their control

12:30 – 13:30 Lunch Break

13:30 – 14:40 **Pathology session 5:** Clubroot and its control

14:40 – 15:00 Coffee Break

ENTOMOLOGY Sessions

10:30 – 12:30 **Entomology session 4:** Agronomy & landscape effects

12:30 – 13:30 LUNCH Break

13:30 – 14:10 **Entomology Session 5:** Sensors for IPM in OSR

14 :10 –14 :30 Discussion Day 2

14:30 – 15:00 Coffee Break

15:00 – 16:00 **JOINT session**

short summary of each session by the chairperson

16:00 – 16:30 **Closing session**, summary by sub-convenors

Poster awards

Presentation of the next meeting site

ZOOM drink and talk together

PROGRAM Overview

PLENARY Session (Pages 12-15)

Chair: Régine Delourme

PS-1: Agriculture in Ukraine with a focus on oilseed crops

Karolina PAWLAK and Arkadiusz SADOWSKI

PS-2: Overview of oilseed rape cultivation in France: cultivation and pest control

David GOUACHE

PS-3: Quantitative genetics and functional genomics of specialized metabolism in *Brassica napus* as a tool for the understanding of biotic interactions

Antoine GRAVOT

PATHOLOGY Sessions (Pages 16-39)

ORAL PRESENTATIONS

Session 1: Blackleg, update on *L. maculans* populations

Chair: Bruce Fitt

S1-1: Characterisation of *Leptosphaeria maculans*, the causal agent of blackleg disease in Northern and Central regions of Germany

Dima ALNAJAR

S1-2: *Leptosphaeria* species causing blackleg disease in oilseed rape and *L. maculans* race incidence in the Czech Republic

Lenka BURKETOVA

S1-3: Race structure characterization of *Leptosphaeria maculans* causing oilseed rape blackleg disease in Tunisia from 2018 to 2020

Essia MAGHREBI

S1.4: Large-scale population survey of *Leptosphaeria maculans* in France highlights both on-going breakdowns and potentially efficient resistance genes in rapeseed

Marie-Hélène BALESSENT

Session 2: Blackleg Resistance

Chair: Marie-Hélène Balesdent

S2-1: Gene-for-gene interactions contribute to stem canker quantitative resistance in rapeseed

Thierry ROUXEL

S2-2: Characterization of epistatic interactions within a family of structurally conserved fungal effectors and of their broad-spectrum recognition by a plant resistance protein

Nacera TALBI

S2-3: Use of different strategies to enable an optimal introgression of *Brassica nigra* *Rlm10* blackleg resistance gene into *B. napus*

Anne-Marie CHEVRE

Session 3: Diversity of blackleg fungi & associated mycobiota

Chair: Nazanin Zamani Noor

S3-1: Changes in *Leptosphaeria maculans* associated mycobiota on different *Brassica napus* cultivars and over a cropping season, with a focus on fungal pathogens

Mathilde GORSE

S3-2: *Plenodomus biglobosus* can utilize more carbon sources than *P. lingam*

Malgorzata JEDRYCZKA

Overall discussion on blackleg

Moderated by **Nazanin Zamani Noor** and **Thierry Rouxel**

Session 4: OSR diseases and their control

Chair: Birger Koopmann

S4-1: Cultural characteristics and differentiation in virulence pattern of *Sclerotinia sclerotiorum* field isolates

Nazanin ZAMANI NOOR

S4-2: Overview of the joint research project "SkleroPro" - Evaluation of environmental factors affecting *Sclerotinia sclerotiorum*

Sinja BRAND

S4-3: Bacterial volatile mediated dormancy of microsclerotia of *Verticillium longisporum* in soil

Sarenqimuge SARENQIMUGE

S4-4: Azole fungicides sensitivity screening of Irish *Pyrenopeziza brassicae* populations and investigation of molecular mechanisms of insensitivity within *CYP51* gene

Diana BUCUR

S4-5: Systematic map of plant protection management methods in oilseed rape

Anna BERLIN

Session 5: Clubroot and its control

Chair: Ann Charlotte Wallenhammar

S5-1: Disease reaction of different plant species against virulent isolates of *Plasmodiophora brassicae*

Nazanin ZAMANI NOOR

S5-2: Bulk segregant analysis (BSA) for identification of clubroot resistance loci in the rapeseed DH population resynthesis S101 x Raptor

Nazanin ZAMANI NOOR

S5-3: Innovative biocontrol measures against *Plasmodiophora brassicae* Wor.

Pratik DOSHI

POSTERS

Chair: Lenka Burketova

P-1: *Leptosphaeria biglobosa* inhibits production of secondary metabolite sirodesmin PL by *L. maculans* in planta

Evren BINGOL – FLASH TALK -

P-2: Occurrence of blackleg pathogens in Swedish winter oilseed rape revealed by Loop-mediated Isothermal Amplification

Zahra Saad OMER – FLASH TALK -

P-3: Marker assisted selection in *Brassica napus* breeding for stem canker (*Leptosphaeria* spp.) resistance

Janetta NIEMANN – FLASH TALK -

P-4: Effects of plant age and inoculum concentration on light leaf spot disease phenotypes on oilseed rape

Laura SAPELLI – FLASH TALK -

P-5: Spectrum of resistance to *Verticillium* wilt in Polish WOSR breeding materials

Joanna KACZMAREK – FLASH TALK -

P-6: Investigating mycovirus-mediated systemic resistance in oilseed rape

Jacob LOCKE-GOTEL – FLASH TALK -

ENTOMOLOGY Sessions (Pages 40-76)

ORAL PRESENTATIONS

Session 1 - Insecticides: pyrethroid resistance and alternatives

Chair: Sam Cook

S1-1: Pyrethroid resistance monitoring in French Coleoptera populations in oilseed rape

Laurent RUCK

S1-2: Parasitoid's vertical distribution in flowering oilseed rape and implications for insecticide application

Johannes HAUSMANN

S1-3: Prospects for research on RNAi-based protection of oilseed rape

Jonathan WILLOW

S1-4: Laboratory assessment of the potential of biopesticides to control the cabbage stem flea beetle

Claire HOARAU

Posters: P1-P4

Session 2 – Plant breeding for resistance to insect pests & identification of traits

Chair: Maxime Hervé

S2-1: Perspectives for integrated insect pest protection in oilseed rape breeding

Christian OBERMEIER

S2-2: Evaluation of varietal differences in vigour and tolerance to cabbage stem flea beetle larvae among rapeseed varieties in France

Arnaud VAN BOXSOM

S2-3: Phenotyping of *Brassica juncea-fruticulosa* introgression lines for resistance against *Lipaphis erysimi* aphids

Neha PANWAR

S2-4: Of glucosinolates and saponins – Pollen beetles can cope with general but not specific plant defence compounds in crucifers

Nadine AUSTEL

S2-5: Chemical basis of host-plant rejection of flower buds by a pollinivorous insect

Laura BELLEC

Posters: P5-P7

Session 3 - Behaviour & ecology of OSR pests: towards IPM

Chair: Eve Veromann

S3-1: Understanding how oilseed rape crops are colonized by the cabbage stem flea beetle

Margot TIXERONT

S3-2: Regular and synchronous population cycles of cabbage stem flea beetle facilitate integrated pest management

Ola LUNDIN

S3-3: Insights into aestivation in cabbage stem flea beetle

Gözde GÜNEY and Daniel RÜDE

S3-4: Ecology and distribution of cabbage stem flea beetle and its parasitoids in UK: steps towards IPM

Patricia ORTEGA-RAMOS

S3-5: Assessing cabbage stem flea beetle and rape winter stem weevil risk by integrating agronomic and pest risk

Céline ROBERT

S3-6: Control of *Ceutorhynchus napi* and *C. pallidactylus*

Meike BRANDES

Posters: P8-P10

Session 4 - Agronomy & landscape effects

Chair: Céline Robert

S4-1: Combining agronomy and conservation biological control at a territory scale for pest management

Nicolas CERRUTTI

S4-2: When intercropping also rhymes with insect pest management: the case study of oilseed rape

Ivan HILTPOLD

S4-3: Flower strips do not constitute an aestivation site for cabbage stem flea beetles, in contrast to woodland edges

Justine PIGOT

S4-4: Effects of perennial flower strips on the parasitism of oilseed rape pests

Antoine GARDARIN

S4-5: Spatiotemporal distance between oilseed rape fields reduces pollen beetle abundance without affecting biocontrol

Silva SULG

S4-6: Waiver of oilseed rape production in a large area for one year to reduce insect pest pressure in the following season?

Udo HEIMBACH

Posters: P11

Session 5 – Sensors for IPM in OSR

Chair: Ivan Juran

S5-1: Identification of predators of oilseed rape pests and quantification of predation services using camera trapping

Gaëtan SEIMANDI-CORDA

S5-2: Dynamics of pollen beetle immigration in European oilseed rape

Emily BICK

POSTERS

Chair: Anne-Marie Cortesero

P-1: Incidence of resistance to pyrethroids and carbamates in Czech populations of green peach aphid (*Myzus persicae*) from oilseed rape fields

Tomáš HOVORKA

P-2: Sensitivity of cabbage stem flea beetles to insecticides

Ivan JURAN – FLASH TALK -

P-3: The impact of insecticide applications on the mortality of pollen beetle larvae and parasitism in winter oilseed rape buds

Jaroslav ŠAFÁŘ & Marek SEIDENGLANZ

P-4: Assessing the effect of six essential oils on cabbage seedpod weevil and the parasitoid *Nasonia vitipennis*

Triin KALLAVUS – FLASH TALK -

P-5: Relative susceptibility of canola and non-canola cultivars to *Lipaphis eryimi* aphids

Sathya THIRUMURUGAN – FLASH TALK -

P-6: Developing oilseed rape resistance to feeding by *Psylliodes chrysocephala*: bioassay and considerations

Daniel RÜDE – FLASH TALK –

P-7: Identification of a palatable white mustard variety to cabbage stem flea beetle feeding

Lucy THURSFIELD – FLASH TALK -

P-8: Monitoring cabbage stem flea beetle populations on an oilseed rape field during winter 2021/22

Udo HEIMBACH – FLASH TALK -

P-9: Effect of leaf area loss and introduction of *Psylliodes chrysocephala* larvae on oilseed rape growth and yield

Duncan COSTON – FLASH TALK –

P-10: Long-term trends in *Brassicogethes aeneus* abundance in the UK.

Chris SHORTALL – FLASH TALK -

P-11: The benefits and risks of including Brassicaceous plants in field margins to promote conservation biocontrol of specialist oilseed rape pests

Sam COOK – FLASH TALK -

P-12: Developmental and enzymatic defense response of hemp aphid *Phorodon cannabis* to selected abiotic stresses

Beata BOROWIAK-SOBKOWIAK

P-13: Effect of herbicides and aphids on the synthesis of fatty acids in *Cannabis sativa*

Roma DURAK

PLENARY Session

PS-1: Agriculture in Ukraine with a focus on oilseed crops

K. Pawlak, A. Sadowski

Department of Economics and Economic Policy in Agribusiness, Poznań University of Life Sciences, Poznań, Poland

Due to the relatively large area and one of the best soil types all around the world Ukraine is a big producer of agricultural products, before all grains and oilseed crops. For this reason it is also one of the key exporters of those commodities. The aim of the paper is to present the importance of the Ukrainian agricultural sector to the world market with special emphasis on grain and oilseed crops. Production capacity measured with agricultural land area, as well as the volume of plant production and trade flows will be discussed. The study uses the data from FAOSTAT [1] and UNCTAD [2] databases.

Ukraine has a great potential for agricultural production. Agricultural area is about 41 million hectares, i.e. more than 68% of total country's surface. Grain production in Ukraine is about 75 million tons per year, which is about 2.5% of world production. Ukraine is also a significant producer of oilcrops, especially sunflower. Quantity of production of this plant material is about 13 mln of tons, which is more than 26% of global production. At the same time Ukraine is the fifth world exporter of cereals and cereal preparations and ranks seventh in export of oilseeds. It is also the third world exporter of vegetable oils, right after Indonesia and Malaysia. In 2020 the values of Ukrainian export of those commodities were around 9.8 billion USD, 1.8 billion USD and 5.7 billion USD respectively, while the shares of that country in global export amounted to 5%, 2% and 7%. Ukraine plays a strategic role in the world export of wheat, barley, maize, rapeseed and sunflower oil. In 2020 export of the above-mentioned plant crops from Ukraine made around 10% of the world export, while in case of sunflower oil the share of Ukraine in global export exceeded 40% both in quantitative and value terms. European and Asian economies, as well as Northern African countries are major destination markets for those products.

Although Ukraine is not a large player in the world agri-food trade (its share in the world exports is only 1.4%, while in imports it amounts 0.4%), it is an important supplier of cereals, rapeseed and sunflower oil. The supply of these products plays a decisive role in ensuring food security of many countries in Africa and the Middle East. It is estimated by the Ukrainian Ministry of Agriculture that as a result of the military conflict between Russia and Ukraine, in 2022 the acreage sown in spring crops in Ukraine may be even 50% smaller than in 2021 [3]. This may result in a suspension or a significant reduction of Ukrainian agricultural exports, while losses in grain export revenue may reach 6 billion USD [4].

Keywords: Ukraine, agricultural production, agricultural trade, world market, grains, oilseed crops

References:

[1] <http://faostat.fao.org/>

[2] <https://unctadstat.unctad.org/wds/ReportFolders/reportFolders.aspx>

[3] <https://www.reuters.com/world/europe/exclusive-ukraine-2022-spring-crop-sowing-area-could-be-halved-minister-2022-03-22/>

[4] <https://www.reuters.com/business/ukraine-could-lose-6-bln-grain-exports-with-ports-blocked-2022-03-21/>

PS-2: Overview of integrated crop protection in French rapeseed production

C. Robert¹, L. Ruck², A. Lellahi³, F. Duroueix⁴, D. Gouache⁵

¹ *Genetics & Crop Protection Department, Terres Inovia, Le Rheu, France*

² *Transfer & Regional Action Department, Terres Inovia, Chalons-en-Champagne, France*

³ *Transfer & Regional Action Department, Terres Inovia, Agen, France*

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Winter rapeseed is the principal french oilseed crop, and main break crop in cereal dominated cropping systems. Rapeseed's historical growth, reaching 1.5 Mha cropping area and 5 MT production, is the key contributor to France's reduced dependance to imported soybean meals required to feed its livestock, and is underpinned by a strong downstream industry that maximises the use an value of rapeseed oil in both food and biofuel industries. We will present an overview of the relative importance of pest and disease threats to rapeseed production, accross time and space, highlighting 2 diverging tendencies for diseases and pests. Disease threats can be deemed under control, in large part due to strong research in genetics translating into improved varieties (blackleg, crown gall, TuYV, and sclerotinia). Conversely, pests have become a major threat, especially in early stages of the crop cycle. This situation has induced massive losses, up to a third, in cropped area and production, in recent years. We will illustrate the specificities of the french situation regarding cabbage stem flea beetle (*Psylliodes chrysocephala*) and rape winter wtem weevil (*Ceutorhynchus picitarsis*), in which the combination of climate change, regulatory policy, and insect biology [1] have created a "perfect storm". Consequently, the current approach to rapeseed protection in France is based on a very intensive use of agronomical and agroecological control methods, including companion cropping, stimulating crop growth via fertilisation and sowing, vigorous and resistant varieties, combined into an integrated approach with decision rules involving combined crop and pest monitoring [2] [3]. The recent loss of a key active ingredient, phosmet, has created a tipping point for the industry, and requires an even more massive effort mobilising all possible protection techniques.

Keywords: winter rapeseed, France, integrated crop protection, *Psylliodes chrysocephala*, *Ceutorhynchus picitarsis*.

References:

- [1] Bothorel S., et al., 2018. 'Resistance to pyrethroid insecticides in cabbage stem flea beetle (*Psylliodes chrysocephala*) and rape winter stem weevil (*Ceutorhynchus picitarsis*) populations in France', *IOBC-WPRS Bulletin*, 89–104.
- [2] Cadoux, S. et al., 2015. *Intercropping frost-sensitive legume crops with winter oilseed rape reduces weed competition, insect damage, and improves nitrogen use efficiency*, *OCL*, 22(3).
- [3] Robert, C. et al., 2021. *Use of agroecological strategies to control rape winter stem weevil and cabbage stem flea beetle damage in winter oilseed rape*. *GCIRC Technical Meeting*

PS-3: Quantitative genetics and functional genomics of specialized metabolism in *Brassica napus* as a tool for the understanding of biotic interactions

A. Gravot et al.

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Specialized metabolites play major roles in plant-environment interactions. A key challenge is extending the knowledge of plant specialized metabolite (SM) diversity and identifying genomic determinants controlling their biosynthesis. Our current works combine metabolomics and genomic approaches to investigate the constitutive and inducible phytochemical diversity in *Brassica napus*, its genetic control, and its possible roles in biotic interactions.

An in-depth mass-spectrometry analysis of root and leaf phytochemical contents revealed 36 glucosinolates (some of which rarely reported in *Brassica* species), 32 phenolics and few additional unknown compounds were purified and structurally investigated by RMN. The quantitative profiling of those compounds in a panel of 281 accessions highlighted the existence of large phytochemical contrasts among varieties. The ratio between leaf contents of sinapoyl-O-hexosides and kaempferol-O-triosides defined two main oilseed rape chemotypes. Based on this large phytochemical dataset, the genetic architecture of phytochemical profiles in leaves and roots was investigated by GWAS. The resulting 89 identified mQTL were organized in series of modules, each controlling different sub-groups of glucosinolates and phenolic compounds.

These results offer tools to understand the impact of breeding selection history on the phytochemical composition of modern varieties, and open perspective for the breeding of any metabolic traits of possible ecological significance in *B. napus*. Beyond intra-specific diversity, we also present our current work to extend the range of the specialized metabolite and defense repertoire of *B. napus*, by introgressing (in the oilseed rape accession 'Aviso') the phytochemical diversity from a 20 accession-panel of the diploid parental species *B. rapa* and *B. oleracea*.

Keywords: genetical metabolomics, specialized metabolism, *Brassica napus*, biotic interactions

PATHOLOGY Sessions

S1-1: Characterisation of *Leptosphaeria maculans*, the causal agent of blackleg disease in Northern and Central regions of Germany

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University of Goettingen, Goettingen, Germany*

Leptosphaeria maculans, the causal agent of blackleg disease, is one of the major pathogens of oilseed rape (*B. napus*) in Germany. Using resistant cultivars is one of the most sustainable and effective control methods. Two types of resistance are available: qualitative resistance and quantitative resistance. Qualitative resistance depends on major resistance genes whose efficacies are related to the frequencies of the corresponding avirulence (*Avr*) genes in a *L. maculans* population.

In this work, we characterised different populations of the phytopathogen *L. maculans* by their races in Northern and Central regions of Germany. Additionally, the ratio of *L. maculans* mating types in each population was checked. Characterization of 11 *Avr* genes in 574 isolates was conducted using either *B. napus* differential set or avirulence gene specific PCR markers. Fifty-two races were described, among which *AvrIm6*, *-7*, *-11*, *AvrlepR1*, *-R2* was the dominant. The findings of this study conclude that *Rlm6*, *Rlm7*, *Rlm11*, *LepR1* and *LepR2* can still provide effective resistance to blackleg disease in German fields. Nonetheless, a cautious deployment of these R genes is necessary since the results showed that the virulent isolates to all of them are already present in the *L. maculans* populations in Germany and their frequency can increase within a few years leading to a loss of R gene efficacy. Results showed that some other major R genes such as *Rlm1*, *Rlm3*, *Rlm4* and *LepR3* are partially effective with *Avr*-frequencies in the pathogen populations $\leq 42\%$, while the absence of *AvrIm2* demonstrated that *Rlm2* is 100% ineffective in the regions investigated. Mating types of *L. maculans* population did not deviate from the ratio 1:1. That emphasizes a central role of the sexual reproduction of the pathogen in hastening the overcome of race-specific resistance in Germany. All in all, our study provides important data for the establishment of a deployment strategy of blackleg resistance genes by rotating R gene harboring cultivars.

Keywords: *L. maculans*; *L. biglobosa*; *B. napus*; oilseed rape, blackleg disease, Phoma stem canker, qualitative resistance; major resistance genes

S1-2: *Leptosphaeria* species causing blackleg disease in oilseed rape and *L. maculans* race incidence in the Czech Republic

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Blackleg represents one of the most serious diseases of oilseed rape worldwide. In the Czech Republic, the area sown by oilseed rape is steadily increasing. Avirulence genes (*AvrLm*) have been identified in *L. maculans* and some of them were cloned. Their counterparts 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 [1, 2].

485 isolates were collected in the autumn both in commercial and trap cultivars from 2017-2020. Cotyledons and true leaves with necrotic spots with pycnidia were used for isolation of single-spore isolates. To distinguish *L. maculans* from *L. biglobosa*, PCR analysis are used [3]. The race spectra avirulence genes were 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 [2].

Incidency of *L. maculans* and *L. biglobosa* were collected in 17 localities in Czech Republic. Total number of *L. maculans* races was 17. The dominant race of *L. maculans* was *AvrLm7*, that was found in 83.92% of cases, corresponding to 407 *L. maculans* isolates. Other races were minority compared to the previous race. The next most represented race was *AvrLm3*, which was found in 4.95%, ie 24 isolates, followed by *AvrLm1AvrLm7*, which was identified in 2.89%, ie 14 tested isolates. 10 isolates of 485 total isolates were not found avirulence genes. This finding corresponded to 2.06% of *L. maculans* races.

Keywords: phoma stem canker; blackleg; oilseed rape; avirulence genes; resistance genes

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References:

- [1] Balesdent, M.H. et al., 2006. A large-scale survey of races of *Leptosphaeria maculans* occurring on oilseed rape in France. *Eur. J. Plant Pathol* 114, p. 53.
- [2] Mazáková, J. et al., 2017. Analysis of *Leptosphaeria* species complex causing phoma leaf spot and stem canker of winter oilseed rape (*Brassica napus*) in the Czech Republic. *Crop & Pasture Science*, 68, p. 254.
- [3] Winter, M. and Koopmann, B., 2016. Race spectra of *Leptosphaeria maculans*, the causal agent of blackleg disease of oilseed rape, in different geographic regions in northern Germany. *Eur. J. Plant Pathol.*, p. 629.

S1-3: Race structure characterization of *Leptosphaeria maculans* causing oilseed rape blackleg disease in Tunisia from 2018 to 2020

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Stem blackleg of oilseed rape (*Brassica napus* L.) caused by *Leptosphaeria maculans* Desm. Ces and de Not. (*L. maculans*), anamorph of the *Phoma lingam*, has become one of the most damaging disease worldwide [1]. In Tunisia, the first appearance of *L. maculans* has been reported on wild radish (*Raphanus raphanistrum* L.) in 2009 [2]. Recently, *L. maculans* complex species has been afresh identified in oilseed rape in Tunisia, since the reintroduction and cultivation increase of this crop in 2014. With a variable range of severity and incidence, the blackleg disease is becoming a threatening issue in Tunisian fields.

Thus, in this study we aimed to report the presence of this phytopathogenic fungus in Tunisia and to describe its race diversity using a set of differential hosts carrying 10 major resistance genes (*Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm7*, *Rlm9*, *LepR1*, *LepR2* and *LepR3*). In total, 146 Tunisian isolates collected from three successive cropping seasons (2018, 2019 and 2020) were characterized. The experiments were conducted in controlled conditions in the Department of Crop Sciences related to the Georg-August-University of Göttingen (Germany). Nine cotyledons for each host line were 4 lobe-wounded and then inoculated with 10 µl of spore suspension adjusted to 1.10⁷ spores/ml [3]. The scoring has been held 14 days-post inoculation using the IMAScore scale [4].

The results revealed the presence of 10 *L. maculans* races. The presence of *L. biglobosa* was also confirmed and represented almost 22% of the collection. All *L. maculans* isolates carried virulence to *Rlm2*, *Rlm3* and *Rlm9*. However, *Rlm7* and *LepR1* resistance genes have not been overcome yet by the Tunisian *L. maculans* population.

In conclusion, our study showed the relatively high race diversity of *L. maculans* complex species in Tunisia. This finding should improve our understanding on this newly emerged fungus and better guide the resistance management strategy of blackleg disease in Tunisia.

Keywords: *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, oilseed rape, race, resistance.

References:

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S1-4: Large-scale population survey of *Leptosphaeria maculans* in France highlights both on-going breakdowns and potentially efficient resistance genes in rapeseed

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Leptosphaeria maculans, the stem canker agent of rapeseed, develops gene-for-gene interactions with its host, and shows a high evolutionary potential to “breakdown” novel resistance genes (R, *Rlm*) deployed on large areas [1]. For an optimal management of R genes, updated knowledge of the population structure of the pathogen is needed. In France, large scale surveys were performed at 10 years intervals since 2000 [2].

Here we report the characterization of a large *L. maculans* population collected in France in 2019-2020. For that purpose, a rapeseed variety devoid of R genes was sown in the fields to be surveyed. Single-pycnidium isolates were recovered from individual leaf spots developed on this variety. Around 80 isolates per site were thus recovered, purified, and characterized using a standard inoculation test on a differential set with different R genes [2]. This enabled us to estimate the frequency of avirulent isolates toward eight R genes.

A total of 832 isolates were collected from 11 sites scattered in 10 French departments. All isolates were virulent toward *Rlm2* and *Rlm9*, as previously described [2]. Very few isolates were avirulent on *Rlm1* (1%, all from the “Nord” department) and *Rlm4* (0.2%). Avirulent isolates toward *Rlm7* varied from 67% to 11.3%, depending on the site sampled, illustrating the ongoing breakdown of *Rlm7*. As previously observed [3], the decrease of *AvrLm7* isolates (29.2% at the national level) compared to the 2010 survey (96.5%) was accompanied by an increase of avirulent isolates on *Rlm3* (0% in 2010; 50.8% in 2019). However, virulent isolates on both *Rlm3* and *Rlm7*, previously rarely detected, were now found in all sites with a mean frequency of 20%. Finally, most or all isolates were avirulent on three R genes, *Rlm11*, *LepR2 (Rlm5)* and *Rlm6*, suggesting their potential efficiency to control the disease. These data will help the reasoned construction and use of resistant rapeseed varieties in France. Funding: CASDAR project C2018-11 (Atypical)

Keywords: Phoma stem canker, rapeseed, avirulence gene, evolution, resistance breakdown, *Rlm7*, *Rlm3*, resistance management, survey.

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S2-1: Gene-for-gene interactions contribute to stem canker quantitative resistance in rapeseed

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The most environmentally friendly way to control plant pathogens involves the use of genetic resistance. "Quantitative" resistance has great advantages in terms of sustainability, but its genetic or molecular mechanisms are poorly known, making it difficult to identify or select. To better control stem canker of *Brassica napus*, caused by the fungus *Leptosphaeria maculans*, we designed an original strategy to validate the hypothesis that (at least) part of the quantitative resistance would involve "gene-for-gene" interactions.

Classically, we identify resistance genes in genotype collections via a rapid, reproducible and miniaturized inoculation test on cotyledons. In contrast, the screening of genotypes to identify resistance at the adult stage of plants is difficult to perform (lack of reproducibility, small number of plants that can be tested, duration of the screening). To overcome this difficulty, we forced the expression of ten selected "late" effectors of the fungus so that the mutant strains express them at the cotyledon stage. Using such strains, we screened a worldwide collection of genotypes representative of the diversity of *B. napus*.

We thus firstly identified a semi-winter Asian oilseed rape genotype that recognizes a late effector, LmSTEE98 during cotyledon infection and showed that this interaction limits the stem canker development. We determined that a single gene controls the resistance and we genetically mapped the corresponding gene, called *RImSTEE98*. Additional semi-winter genotypes were identified that reacted to LmSTEE98, or to another "late" effector LmSTEE6826. In addition, five winter-type genotypes displayed an intermediate response to another late effector, LmSTEE7919. These results strongly suggest that gene-for-gene interactions could operate in the stem to contribute at least partially to quantitative resistance and that the strategy we designed allows the identification of new sources of resistance in a model that is sorely lacking them.

Keywords: Phoma stem canker, rapeseed, effector, manipulation of effector expression, quantitative resistance, diversity panel.

S2-2: Characterization of epistatic interactions within a family of structurally conserved fungal effectors and of their broad-spectrum recognition by a plant resistance protein

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Recognition of a pathogen avirulence (AVR) effector protein by a cognate plant resistance (R) protein triggers a set of immune responses that render the plant resistant. Pathogens can escape this resistance by different mechanisms including the acquisition of new effectors that suppress AVR recognition. *Leptosphaeria maculans*, causal agent of oilseed rape stem canker, develops a high diversity of mechanisms to efficiently escape plant resistance including suppression of R protein-mediated recognition by an AVR effector. Indeed, *AvrLm4-7* suppresses the recognition of *AvrLm3* and *AvrLm5-9* by the R proteins *Rlm3* and *Rlm9*, respectively [1,2]. To decipher the epistatic interaction between these *L. maculans* AVR effectors, we determined the crystal structure of *AvrLm5-9* and obtained a 3D model of *AvrLm3*, based on the crystal structure of *Ecp11-1*, a homologous AVR effector from *Fulvia fulva*. Despite a lack of sequence similarity, *AvrLm5-9* and *AvrLm3* are structural analogues of *AvrLm4-7* (structure previously characterized [3]). Structure-informed sequence database searches identified a larger number of putative structural analogues among *L. maculans* effector candidates, including *AvrLmS-Lep2*, as well as among effector candidates from other phytopathogenic fungi [4]. Remarkably, transformants of *L. maculans* expressing one of these structural analogues, *Ecp11-1*, triggered *Rlm3*-mediated immunity. Furthermore, this recognition was suppressed by *AvrLm4-7*. On these bases, we continued to decipher the interactions between *Ecp11-1* / *AvrLm3* (and their closely related homologues) and *Rlm3* by: (i) performing *L. maculans* complementation experiments with different homologues; (ii) determining whether *AvrLm4-7* could suppress that recognition; (iii) finely characterizing the amino-acids / regions of *Ecp11-1* and *AvrLm3* which induced recognition by *Rlm3*. This analysis is a first step towards the understanding of broad-spectrum resistances that may allow multi-pathogen disease management.

Keywords: *Leptosphaeria maculans*, oilseed rape, avirulence, 3-D structure, evolution, specific resistance, broad-spectrum resistance.

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S2-3: Use of different strategies to enable an optimal Introgression of *Brassica nigra* *Rlm10* blackleg resistance gene into *B. napus*

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Blackleg disease, caused by *Leptosphaeria maculans*, leads to heavy losses in rapeseed production. Its impact is contained by *L. maculans* resistance gene (*Rlm*) diversification through resistant varieties breeding [1]. One highly efficient gene, *Rlm10*, was identified on B04 chromosome of *Brassica nigra* (BB, 2n=16) from *B. napus*-*B. nigra* monosomic addition lines (AACC+1B chromosome, 2n=39) [2]. Thereafter, it was introduced on the A09 chromosome of rapeseed (AACC, 2n=38) but with a very large introgressed region of the B04 chromosome. This confers a poor agronomic value to *Rlm10* homozygous plants. The current challenge is to promote homeologous recombination in order to reduce the size of the B04 introgression carrying *Rlm10* in *B. napus*.

For that purpose, we used the addition line (AACC+B04, 2n=39) and the introgressed resistant line (AACC, 2n=38) to generate plants with different genomic structures that favor homeologous pairing. To identify the best strategy, the B04 chromosome was placed either in an AC haploid state, the ACB04 (n=20) haploid plant producing unreduced gametes, or in triploid state with only one set of C chromosomes, AACB04 (2n=30). Additionally, AAC (2n=29) plants with the B04 introgression were produced as we know that homologous pairing is highly promoted between A chromosomes in such hybrid structure [3, 4].

The pairing events were quantified in metaphase I. This analysis was made possible by the multicolor fluorescent labelling of meiotic chromosome spreads. We use Genomic In Situ Hybridization (GISH) and GISH-like, to discriminate each *Brassica* subgenomes (A, C and B04). Our preliminary results show that the triploid structures, with the introgression or the B04 addition, are the most likely to produce recombination events between the A/C and B genomes. Later, KASPar (Kompetitive Allele Specific PCR) molecular markers designed along the B04 chromosome will allow identifying recombinant plants with reduced introgression size in progenies.

Keywords: Blackleg, resistance genes, homeologous recombination, molecular markers

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S3-1: Changes in *Leptosphaeria maculans* associated mycobiota on different *Brassica napus* cultivars and over a cropping season, with a focus on fungal pathogens

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The rapeseed pathogen *Leptosphaeria maculans* is mainly controlled by the use of varieties with specific resistance genes (*Rlm*) and/or quantitative resistance. The influence of resistant genotypes of the host plant on the targeted pathogen is obvious, however, the pathogen is now considered in a more global environment, interacting with the whole microbiota present locally. Thanks to the development of new technologies, we are now able to describe the microbiota associated with a pathogen and to analyze how it can be modified by the plant genetic background or during a growing season.

Here, we used metabarcoding sequencing of two barcode genes used for fungal identification (Internal Transcribed Spacer (ITS) region and the single-copy gene Actin) in order to describe the mycobiota present on rapeseed field samples (leaves or stem) at five key time points of the *L. maculans* life cycle over the cultural season [1]. Our study was performed on rapeseed varieties carrying different resistance genes (none, quantitative resistance, *Rlm11* [2] or *RlmS* [3]). The whole data set demonstrated the complementarity of ITS and Actin genes for such studies.

Our preliminary results on susceptible cultivars illustrated that metabarcoding is a powerful method to identify and quantify species including the main rapeseed fungal pathogens. Their evolution over one growing season fits our current knowledge on their epidemiology. We also evaluated the response of the main rapeseed pathogens to the resistance present in the selected varieties. Finally, focusing on two genotypes, we detected a strong year effect on the distribution of fungal species. Visual symptom identification remains difficult for non-experts. Our data suggest that metabarcoding could be a complementary tool targeting many pathogens at the same time, when there is a precise identification need to answer specific biological questions or to evaluate resistance toward numerous diseases.

Keywords: Rapeseed; *Leptosphaeria maculans*; diversity; pathogens; metabarcoding; ITS; Actin.

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S3-2: *Plenodomus biglobosus* can utilize more carbon sources than *P. lingam*

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Until 2011 *Plenodomus biglobosus* and *P. lingam* were classified as one species, called *Leptosphaeria maculans*. Then, *L. biglobosa* has been separated from *L. maculans* based on morphological characters [1], what was also supported with numerous chemical, biochemical and genetic studies. Recently, these two species have been renamed to *P. biglobosus* and *P. lingam*. They have a similar life cycle but *P. biglobosus* is found more often and it can infect a broader range of plants. Both first infect leaves of host plants, including oilseed rape (*Brassica napus*) and then overgrow veins and reach the stem via leaf petioles. *Plenodomus lingam* reaches the stem cortex, whereas *P. biglobosus* usually keeps closer to the outer tissues (epidermis and sub-epidermis). The latter species is also frequently found in inner parts of the stem. In this study we hypothesized the metabolic capacities of *P. lingam* and *P. biglobosus* vary to a great extent.

To test this hypothesis we have grown both species in the wells of FF microplates (Biolog) containing 95 carbon sources and tetrazolium dye. There were three isolates per species and three technical replicates. We have calculated the number of utilized substrates and the efficiency of their use. It was found that substrate richness, calculated as the number of utilized substrates and the number of substrates allowing effective growth of the isolates showed significant differences between these two species. *Plenodomus biglobosus* was able to utilize 60 substrates, and 34–48 carbon sources were used very efficiently. Cluster analysis based on Sneath criteria divided *P. biglobosus* into two groups. In contrast the most effective isolate of *P. lingam* utilized 36 carbon sources, 25–29 were used very efficiently and all isolates of *P. lingam* formed one group.

We claim the similarities allow both species coexist on the same host plant but, among other characters, the differences in utilization of carbon sources contribute to different growth habits.

Keywords: carbon sources, cluster analysis, coexistence, FF microplate, substrate utilization;

References:

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S4-1: Cultural characteristics and differentiation in virulence pattern of *Sclerotinia sclerotiorum* field isolates

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Sclerotinia stem rot (SSR) or white mould, caused by *Sclerotinia sclerotiorum*, is a major threat to oilseed rape production in Germany and may cause significant yield losses if not controlled by cultural management strategies and fungicide applications (Zamani-Noor 2021). The aim of current study was to evaluate the variabilities in morphological characteristics and pathogenicity factors in *S. sclerotiorum* population gathered from different regions in Germany.

Fifty-seven isolates of *S. sclerotiorum* were collected during a nationwide Sclerotinia-monitoring program in 2020 and 2021. Physiological and morphological analyses such as the mycelial growth index, mycelial compatibility grouping, shape and colour of colony, the number of produced sclerotia per Petri dish and the average weight of sclerotium were determined. Furthermore, three different methods, a cotyledon test, an adult-plant inoculation as well as a detached leaf bioassay, were conducted to assess aggressiveness of the collected isolates on selected genotypes of oilseed rape cultivars.

Among all *S. sclerotiorum* isolates analysed, high phenotypic and pathogenic variability were observed. While, the colony colour was described as whitish, dirty white, light grey and brown to black, the type of growth was classified as fluffy or sparse. Significant differences ($p \leq 0.05$) were also discovered between the isolates in the average number of sclerotia per Petri dish. Further, significant differences were observed between different isolates ($p \leq 0.05$) in relation to pathogenicity. Regardless of the evaluation method, the isolates could be divided into highly aggressive, moderate, or low aggressive. Differences were also noticed between the different *Brassica* genotypes in their responses to different isolates of *S. sclerotiorum*.

The results of this study contribute new knowledge of variation in cultural characteristics and aggressiveness in the *S. sclerotiorum* population.

Keywords: Oilseed rape, *Brassica napus*, white mould, pathogenicity factors, aggressiveness, mycelial compatibility group, cotyledon test, detached leaf bioassay, disease screening

References:

- [1] Zamani-Noor N., 2021. Baseline sensitivity and control efficacy of various group of fungicides against *Sclerotinia sclerotiorum* in oilseed rape cultivation, *Agronomy*, vol. 11, no. 9, p. 1758.

S4-2: Overview of the joint research project "SkleroPro" - Evaluation of environmental factors affecting *Sclerotinia sclerotiorum*

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Sclerotinia stem rot (SSR) is an economically damaging disease of winter oilseed rape in all areas where oilseed rape cultivation is intensified (Zamani-Noor 2021). SkleroPro, a forecasting model, was designed to support the decision-making process for fungicide application against SSR during the flowering stage (Koch et al. 2007). The current study aims to update and evaluate the environmental data affecting the SkleroPro model.

Climate chamber experiments were designed to evaluate the effect of temperature, light intensity, relative soil humidity, and soil type on carpogenic germination of sclerotia under controlled conditions. Results showed that a temperature of 15 °C at a relative soil humidity of 99% was optimal for sclerotia germination. Additionally, a day/night regime of 12/12 h with a UV light component was necessary to form apothecia. At lower temperatures, the formation of stems and apothecia took significantly longer time. At higher temperatures, stems were thinner and longer. Lower soil moisture resulted in slower germination. No germination could be documented below relative soil moisture less than 50%.

In addition to the experiments under controlled conditions, sclerotia depots were used to monitor the emergence of sclerotia germination and ascospore release periods under field conditions. Each depot consisted of 100 sclerotia buried at 3-5 cm depth in autumn. A data logger was also buried next to the depots to record soil temperature and humidity every half hour. In 2021, the first apothecia were observed on the 10th of May. After that, the germination rate increased to 27% until the 29th of May and then decreased to 2% on the 17th of June. Based on the data from the data logger, it could be shown that 13-15 °C and 55 to 60% soil humidity were essential as minimum conditions for sclerotia germinations.

Acknowledgements: Authors thank the BMEL for financial support.

Keywords: *Oilseed rape, Brassica napus, white mould, ValiProg, forecast model, climate chamber experiment, carpogenic germination, temperature, light intensity, relative humidity, soil moisture*

References:

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- [2] Zamani-Noor N., 2021. Baseline sensitivity and control efficacy of various group of fungicides against *Sclerotinia sclerotiorum* in oilseed rape cultivation, *Agronomy*, vol. 11, no. 9, p. 1758

S4-3: Bacterial volatile mediated dormancy of microsclerotia of *Verticillium longisporum* in soil

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The major oilseed rape pathogen *Verticillium longisporum* forms melanized resting structures, microsclerotia, on its host plants, which are returned into soil with plant straw and stay viable in soil for several years [1] [2]. Since microsclerotia formation is an important survival mechanism of this pathogen, it is of great significance to study the factors involved in microsclerotia dormancy as this may open novel strategies of preventive disease control.

Most fungal species are thought to be affected by soil fungistasis, which is mainly caused by soil microorganisms, particularly bacteria [3] [4]. In this study, microsclerotia were incubated with living bacterial suspension (around 5×10^8 CFU/mL) originated from 21 different bacterial isolates (19 of them were obtained from the bulk soil or rhizosphere of field-grown oilseed rape). The results showed that all tested bacterial isolates had inhibitory effects on microsclerotia germination. A further bioassay in two-compartment petri dishes was conducted to investigate the effect of bacterial volatiles on microsclerotia. The average diameter of the colonies formed surrounding the microsclerotia was dramatically reduced in all bacterial treatments when compared to the control, which confirmed the strong volatile mediated inhibition effect of bacteria on microsclerotia germination. Bacterial volatile compounds of four bacterial isolates were identified using gas chromatography/mass spectrometry. Among 45 identified volatile compounds, two active acid and two alcohol compounds were detected in all four bacterial isolates tested. A bioassay using pure volatile compounds revealed that acidic volatile compounds strongly inhibited microsclerotia germination and colony growth, implying that volatile acidic compounds may be a major factor by which bacteria inhibit microsclerotia germination in the soil.

Keywords: Bacteria, antifungal, dormancy, *Brassica*, soil.

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S4-4: Azole fungicides sensitivity screening of Irish *Pyrenopeziza brassicae* populations and investigation of molecular mechanisms of insensitivity within *CYP51* gene

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Light leaf spot disease of Brassicas (LLS), caused by *Pyrenopeziza brassicae*, is amongst the most damaging diseases of winter oilseed rape (OSR) (*Brassica napus*) and a significant threat to Irish crops. As not much is known about the epidemiology of the disease, control relies upon the use of fungicides, with sterol 14 α -demethylase (*CYP51*) inhibitors (azoles), representing the main class of fungicides used. A shift in the sensitivity towards azole fungicides has been documented for different European *P. brassicae* populations, including a limited population from Ireland [1].

As it is unknown if the results previously published are representative for Ireland, we aimed to determine the degree of sensitivity for the Irish population by assessing the sensitivity of *P. brassicae* isolates from 2019-2020 to tebuconazole and prothioconazole-desthio, and to confirm the presence of the resistance mechanisms within the *CYP51* gene.

A change in sensitivity to azoles was observed in the Irish populations when compared to older *P. brassicae* reference isolates [2], and differences between the populations were present in terms of tebuconazole sensitivity. Two substitutions were identified within *CYP51* (G460S and S508T) and an insert of different sizes in the promoter region. Compared to wild-type isolates, those carrying G460S or S508T were less sensitive to tebuconazole and prothioconazole-desthio, and when the alterations were present with an insert, further reductions in sensitivity were observed.

Keywords: *Pyrenopeziza brassicae*, azoles, fungicide sensitivity, disease control.

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S4-5: Systematic map of plant protection management methods in oilseed rape

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Oilseed rape (OSR; *Brassica napus* L.) is a highly valued crop and during recent decades, its area of production and profitability have increased. Concurrently, several diseases negatively impact OSR production. The aim of this systematic map was to provide an overview of plant protection measures that can be directly implemented in crop production management in OSR production and to identify knowledge gaps and areas where more research is needed. The method used for this systematic map follows the ROSES reporting standard. The search strategy was developed in collaboration with stakeholders and designed to cover available scientific evidence for OSR disease management in climate relevant for Scandinavian crop production.

Searches in five scientific databases collected 4 633 articles and was complimented with additional searches in grey literature sources. Articles were screened at title, abstract and at the full text level, where 897 articles were evaluated. Of these, 118 articles included studies of OSR crops and all measures to control crop disease in agricultural fields and were considered eligible to be included the map. The comparator was no intervention, and outcomes from interventions were determined as yield per unit area, disease suppression or increase in crop quality. Eligible articles were coded in the following categories; location and climate zone, disease, pathogen, intervention and management method, outcome and study design.

The systematic map reports the body of scientific studies on plant disease protection measures in OSR based on field trials. The country (Canada) and region (Europe) with the largest OSR crop production areas also contributed with the highest number of articles. In total, 17 different diseases were reported, black leg being the most studied disease. Nineteen different intervention methods or management types were examined, cultivar resistance and pesticide application were the most commonly studied control measures.

Keywords: *Brassica napus*, rapeseed, canola

S5-1: Disease reaction of different plant species against virulent isolates of *Plasmodiophora brassicae*

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The obligate soilborne pathogen, *Plasmodiophora brassicae* (Pb), is the causal agent of clubroot disease. In recent years, several new clubroot-infested oilseed rape (OSR) fields were detected in Germany (Lüders 2017; Zamani-Noor 2017). It was observed that a threshold of inoculum density of Pb to cause clubroot and proliferate the new resting spores inside the roots highly depended on the pathogen's virulence and the host plant's susceptibility (Zamani-Noor et al. 2021).

In the current study, 86 plant species from 19 botanical families were evaluated for their potential as hosts of Pb. Plants were artificially inoculated with two isolates of Pb, which were either virulent on clubroot-resistant OSR cv. Mentor (P1 (+)) or avirulent on this cultivar (P1). Clubroot severity and the number of resting spores inside the roots were assessed 35 days post inoculation.

Typical clubbed root symptoms were incited only in the Brassicaceae family, none of the other species showed external disease symptoms. Plant species infected with P1 (+) exhibited more severe symptoms, bigger galls and a higher number of resting spores than the plants infected with P1. Among all Brassica species, *Bunias orientalis*, *Coronopus squamatus* and *Raphanus sativus* were fully resistant to both isolates. *Camelina sativa*, *Coincya monensis*, *Descurainia sophia*, *Diplotaxis muralis*, *Erucastrum gallicum*, *Neslia paniculata*, *Sinapis alba*, *S. arvensis*, *Sisymbrium altissimum*, *S. loeselii* and *Thlaspi arvense* were infected severely by both isolates. In contrast, *Conringia orientalis*, *Diplotaxis tenuifolia*, *Hirschfeldia incana*, *Iberis amara*, *Lepidium campestre* and *Neslia paniculata* were completely or partially resistant to P1 but highly susceptible to P1 (+). Furthermore, the pathogen DNA was detected not only in all Brassica species but also in *Alopecurus myosuroides*, *Phacelia tanacetifolia*, *Papaver rhoeas* and *Pisum sativum*. These results suggest that the number and diversity of hosts for Pb are greater than previously reported.

Keywords: Oilseed rape, *Brassica napus*, clubroot, disease severity index, pathogenicity, pathogen virulence, pathotype, aggressive isolates, integrated pest management, host range, cover and catch crops, weeds

References:

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- [2] Zamani-Noor, N., 2017. Variation in pathotypes and virulence of *Plasmodiophora brassicae* populations in Germany, *Plant Pathology*, vol. 66, no. 2, p. 426.
- [3] Zamani-Noor, N. et al., 2021. Greenhouse evaluation of clubroot resistant-*Brassica napus* cv. Mendel and its efficacy concerning virulence and soil inoculum levels of *Plasmodiophora brassicae*, *Pathogens*, vol. 10, no. 2, p. 151.

S5-2: Bulk segregant analysis (BSA) for identification of clubroot resistance loci in the rapeseed DH population resynthesis S101 x Raptor

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Clubroot caused by the biotrophic obligate pathogen *Plasmodiophora brassicae* is a major disease of the *Brassicaceae* family. Genetic resistance can be an effective method for controlling clubroot, but the sources available for resistance to clubroot in oilseed rape are limited.

Resynthesis line S101 has been described as clubroot resistant based on a *Brassica oleracea* C genome-derived resistance (ECD15; Girke 2002). In the current study, a single F1 plant of resynthesis line S101 x Raptor (MSL hybrid cv.) was used to produce doubled haploid (DH) lines. DH population and parental lines were phenotyped in three independent greenhouse experiments against field isolates of *P. brassicae* classified as pathotype P1 (Zamani-Noor 2017). Disease incidence (DI %) and disease severity index (DSI %) were determined 35 days post inoculation (Strelkov et al. 2006). Extreme genotypes were analysed by 15k Illumina Infinium SNP-array. Markers separating resistant and susceptible genotypes were used as KASP markers to genotype remaining DH lines.

The results showed significant differences in DI and DSI between the parents and 120 DH lines. A precise 1 : 1 segregation for DSI <25% and >25% was found, and there was a close correlation between DI and DSI. Furthermore, 18 Illumina 15K SNP markers on chromosome A08 distinguished 55 resistant and susceptible lines. Although clubroot resistance of S101 is derived from the Brassica C genome, no C genome resistance markers were detected.

Our results showed that molecular markers closely linked to the quantitative trait loci (QTL) could be used in marker-assisted selection for plant resistance to clubroot in oilseed rape breeding programs..

Keywords: oilseed rape, *Brassica napus*, *Plasmodiophora brassicae*, disease severity index, pathogenicity, Chi-square test, segregation ratios, quantitative trait loci (QTL), SNP microarray heritability

References:

- [1] Girke, A., 2002. Resynthesized oilseed rape (*Brassica napus* L.) as a new genepool for hybrid breeding. Ph.D. Dissertation, Georg-August-Universität Göttingen.
- [2] Strelkov, S.E. et al., 2006. Characterization of *Plasmodiophora brassicae* populations from Alberta, Canada, *Canadian Journal of Plant Pathology*, vol. 28, p. 467.
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S5-3: Innovative biocontrol measures against *Plasmodiophora brassicae* Wor.

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Plasmodiophora brassicae is one of the most important pathogen of crucifer crops and can have devastating effects resulting in huge loss of yield. Although it is known that the spores germinate to release zoospores that infect the roots, yet, the mechanism that triggers germination of spores is still a mystery. This proposed research will investigate the previously unidentified reason(s) behind germination of spores and to better understand the mechanism to devise appropriate control measures. Additionally, this proposed research will also test combinations of different biocontrol agents against *P. brassicae* resting spores and test their efficiency in controlling the disease.

Keywords: Clubroot, *P. brassicae*, germination, biocontrol

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POSTERS

P-1: *Leptosphaeria biglobosa* inhibits production of secondary metabolite sirodesmin PL by *L. maculans* in planta

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Phoma stem canker is an economically damaging disease of oilseed rape (*Brassica napus*), caused by two co-existing fungal pathogens *Leptosphaeria maculans* and *L. biglobosa*. Interactions between these pathogens affect disease severity. *L. maculans* produces a non-host selective epipolythiodioxopiperazine (ETP) phytotoxin, called sirodesmin PL, allowing the pathogen to have a competitive advantage over other invading pathogens. Results of our recent in vitro studies indicated that simultaneous inoculation with *L. biglobosa* inhibits the production of sirodesmin PL by *L. maculans*. This study aimed to determine whether this inhibition by *L. biglobosa* also occur in planta.

Cotyledons of oilseed rape cultivar Charger were inoculated with *L. maculans* only, *L. biglobosa* only, *L. maculans* and *L. biglobosa* simultaneously, or sterilised distilled water as a control. Secondary metabolites from the lesions were extracted 26 days post inoculation, using ethyl acetate. Through analytical chemistry techniques such as HPLC and LC-MS, the presence of sirodesmin PL was confirmed only in extracts from cotyledons inoculated with *L. maculans* only. These results indicate that *L. biglobosa* inhibits the production of sirodesmin PL by *L. maculans* in planta, if both are inoculated simultaneously.

There is a need to further investigate the mechanisms of this inhibition by *L. biglobosa*. Understanding the interactions between *L. maculans* and *L. biglobosa* can provide new strategies for effective phoma stem canker control, alongside resistant cultivars and fungicide applications.

Keywords: *Leptosphaeria*, sirodesmin PL, interspecific interactions

References:

- Elliott, C. E., Gardiner, D. M., Thomas, G., Cozijnsen, A., van de Wouw, A., & Howlett, B. J., 2007. Production of the toxin sirodesmin PL by *Leptosphaeria maculans* during infection of *Brassica napus*. *Molecular Plant Pathology*, 8(6), 791–802.
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P-2: Occurrence of blackleg pathogens in Swedish winter oilseed rape revealed by Loop-mediated Isothermal Amplification

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Blackleg is a serious disease of *Brassica napus* var. *oleifera* and is caused mainly by the fungus *Leptosphaeria maculans* (Lm), but also to some extent by the species *L. biglobosa* (Lb). The disease is of major economic importance worldwide and causes severe yield losses, especially in winter oil seed rape (OSR) [1]. It is, however, not fully investigated which *Leptosphaeria* species that causes the symptoms in crops of OSR in Sweden. The aim of this study was to investigate the occurrence of these fungi in winter OSR using the previously developed Loop-mediated Isothermal Amplification (LAMP) assays [2].

Plant samples were collected from winter OSR fields in three regions in southern Sweden during 2019-2020 and 2020-2021, respectively. Pooled leaf samples with visual spots were collected in October and infected stems were collected from the same fields in July. DNA was extracted with a commercial kit. Real-time LAMP was performed with Genie® II platform and the occurrence of Lm and Lb was assessed as number of gene copies.

The results showed that both Lm and Lb were prevalent in the sampled fields. *Leptosphaeria maculans* was most prevalent on the leaves in autumn, whereas Lb was most prevalent in the stem samples during summer of both growing seasons. Both species occurred in the stem base and upper part of the stem. There was a difference in the average numbers of gene copies of Lm and Lb between the three regions. Newly started studies include broader nationwide investigations of Lm and Lb in winter OSR as well as identification of optimal temperature for mycelial growth of each species.

Keywords: *Brassica napus*; rapeseed, *Leptosphaeria maculans*; *Leptosphaeria biglobosa*; real-time LAMP

References:

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P-3: Marker assisted selection in *Brassica napus* breeding for stem canker (*Leptosphaeria* ssp.) resistance

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Stem canker of brassicas (blackleg), caused by the fungal complex *Leptosphaeria maculans*-*L. biglobosa* is one of the most destructive diseases of oilseed rape (*Brassica napus*) worldwide. In numerous countries, including Poland both pathogen populations co-exist and they can jointly lead to severe disease symptoms as well as yield losses. Control of this pathogen is heavily dependent on the plant-pathogen interaction and breeding resistant lines.

DNA markers have been used widely in conventional plant breeding programs *via* marker-assisted selection (MAS). However, very few resistance sources against stem canker in *Brassica* species exist, and also the lack of tightly linked molecular markers to these sources restricts the rapid generation of resistant lines. The aim of this study was to use some PCR markers and orthologous genes as markers for screening *Brassica* interspecific hybrids towards increased resistance to blackleg.

The plant material consisted of thirty interspecific *Brassica* progenies and selected parental forms originated from two *Brassica* species *i.e.* *B. napus* and *B. rapa* used for interspecific hybridization. Selected for molecular analysis parental forms were resistant and non-resistant respectively. Based on literature data nineteen specific PCR primers have been used for screening plant resistance/susceptibility. Moreover, 15 orthologous genes were identified for four resistance genes *i.e.* *Rlm1*, *Rlm3*, *Rlm4* and *Rlm7*. These genes were used as new markers for searching interspecific progenies. The plant material was studied using polymerase chain reaction (PCR) and sequencing methods. For this purpose, leaf samples were collected from three individual plants per genotype.

To sum up, most number of tested markers allowed to confirm their effectiveness for identification genotypes with different *Leptosphaeria* resistance genes. However, markers Xbrms075, Fad8, B5-1520, B5Rlm6_1, Bol021435 and BjHZ_1 as well as BnHZ_2 should be verified more precisely.

Acknowledgement: Experimental work was funded by the Ministry of Agriculture and Rural Development of Poland, project number 27.

Key words: resistance gene, *Leptosphaeria* ssp., molecular markers, orthologous genes

References:

[1] Rashid, M. H. et al., 2018. Development of molecular markers linked to the *Leptosphaeria maculans* resistance gene *Rlm6* and inheritance of SCAR and CAPS markers in *Brassica napus* × *Brassica juncea* interspecific hybrids. *Plant Breeding*, 137(3), 402-411.

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P-4: Effects of plant age and inoculum concentration on light leaf spot disease phenotypes on oilseed rape

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Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is one of the most economically damaging diseases of winter oilseed rape (*Brassica napus*) in the UK, with annual yield losses of > £100M. Control of light leaf spot is challenging because it is a polycyclic disease, with epidemics starting in autumn by ascospores. Subsequently, conidia produced by asexual sporulation on infected leaves cause secondary infections on all parts of the plant. Current control relies on fungicides; however, insensitivity development highlights the need for non-chemical controls like host resistance. Host resistance against *P. brassicae* is poorly understood; the aim of this project is therefore to improve our current knowledge by researching virulent races in pathogen populations, identifying candidate resistance genes, and investigating mechanisms of host resistance. This is a HKEP (Hertfordshire Knowledge Exchange Partnership) funded project in collaboration with the Perry Foundation and the industrial partner ADAS.

Effects of plant age, incubation condition and inoculum concentration on light leaf spot disease severity have been investigated using cultivar Charger. *P. brassicae* isolates from the 2020/2021 season were obtained from crop samples through single-spore isolation. Results showed that older plants and higher inoculum concentration produced the most severe symptoms. Additionally, plants that received the higher inoculum (10^5 spores/ml) were significantly shorter by 5 cm than those with lower inoculum concentration (10^4 spores/ml), suggesting possible correlations between fungal inoculum concentration and plant growth. This hypothesis will be investigated in future work. Over 25 *P. brassicae* field isolates have been collected from oilseed rape and kale cultivars across England. Another 60 isolates from continental Europe were obtained through Rothamsted Research. Those isolates will be further screened for virulence.

Keywords: light leaf spot, oilseed rape, *Pyrenopeziza brassicae*, age, inoculum concentration, plant height, disease severity, phenotype

P-5: Spectrum of resistance to *Verticillium* wilt in Polish WOSR breeding materials

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Winter oilseed rape (WOSR, *Brassica napus*) is the main oil crop grown in Poland with cultivation area of 1 M ha in 2021. The increased demand for WOSR and the resulting increase in cultivation area are accompanied by a foreshortening of crop rotations, which causes phytosanitary problems by promoting the spread of fungal diseases. Verticillium wilt is regarded as one of the major agents causing premature ripening and the loss of seed yield. Verticillium wilt is predominantly caused by *Verticillium longisporum* [1]. Resistance of WOSR to *V. longisporum* is difficult to control owing to the absence of efficient fungicides. Since breeding of resistant cultivars currently is the most promising option to control Verticillium wilt, the identification of *B. napus* genotypes with improved resistance against *V. longisporum* is very important.

The aim of this study was to evaluate the level of resistance to Verticillium wilt in Polish WOSR breeding materials. A total of 90 breeding lines of *B. napus* supplied by 3 Polish breeding companies have been tested in 2021 and 2022 (ongoing) in glasshouse conditions. Each line was tested in 20 replicates. Seeds were sown to a mixture of sand and peat under day/night conditions of 16 h/8 h at 20°C/18°C. After 10 days, seedlings were removed from the soil and in such way the root tips were slightly damaged. The root system was gently washed and placed in a conidial suspension (10^6 conidia/ml) for 15 minutes. The development of symptoms was assessed 28 days after inoculation using 0 to 9 scale [2]. Considerable differences were found between breeding lines of WOSR originating from three breeding stations. This result is very promising and suggests that some resistance is already present in the current Polish breeding material of WOSR.

Keywords: *Verticillium longisporum*, premature ripening, resistance, yield loss;

References:

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P-6: Investigating Mycovirus-Mediated Systemic Resistance in Oilseed Rape

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Oilseed rape, *Brassica napus*, is an important and popular food crop for making rapeseed oil, rapeseed meal for cattle, and is used increasingly by the biofuels industry. The crop is attacked by many fungal diseases globally, such diseases cause causing hundreds of millions of pounds worth of yield losses annually in the UK [1], namely light leaf spot (caused by *Pyrenopeziza brassicae*) and phoma stem canker (caused by *Leptosphaeria maculans* and *L. biglobosa*). Previously a quadrivirus (LbQV-1) was characterised that infects *L. biglobosa*, which then interferes with *L. maculans* pathogenicity (Shah et al. 2020). Additionally, plant response genes were differentially expressed to combat enhanced pathogenicity.

My project involves screening and characterising viruses in *L. maculans*, *L. biglobosa* and *P. brassicae* and quantifying how rape recognises and responds to each fungus. Virus-infected and virus-free isogenic lines of the Chinese *L. biglobosa* isolate (W10) have been revived to do challenge assays in planta. Endemic isogenic lines have also been produced. RT-PCR conditions were optimised, and LbQV-1 specific primers used to confirm virus presence previously ascertained by dsRNA isolation. Endemic field isolates were collected and, to date, 26 *L. maculans*, 28 *P. brassicae* and one *L. biglobosa* isolates, and 19 international *P. brassicae* isolates have been screened. Apart from *L. biglobosa* isolate WH17-WHY-1, which contained LbQV-1, none of the other fungi appeared to be virus-infected.

To gain insight into the molecular mechanisms underpinning the observed phenotypes, samples will be collected from different parts of challenge inoculated plants (i.e. stem, leaf, root, petiole etc.) and compared in terms of gene regulation using next-generation sequencing and quantitative PCR.

Keywords: Mycovirus, Oilseed rape, Phoma stem canker, Light leaf spot, fungus–plant interactions, systemic acquired resistance

References:

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ENTOMOLOGY Sessions

S1-1: Pyrethroids resistance monitoring in French coleoptera populations in oilseed rape

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The main rapeseed pests are beetles. For decades, pyrethroids have been the main insecticides used against these beetles. Terres Inovia and its partners in the "Resistance" working group of Vegephyl* followed the resistance evolution of this insecticide family [1].

Today, resistance of pollen beetles (*Brassicogethes* sp.) to pyrethroids is considered widespread. For cabbage stem flea beetle and rape winter stem weevil populations, pyrethroid resistances are well established. Some populations of cabbage stem flea beetle (*Psylliodes chrysocephala* L.) populations present M918L mutations (skdr - super knock-down resistance) which confer a very high level of resistance to pyrethroids. This mutation, historically present in the North-East of France, progresses and has been detected in new departments. Others mechanisms, mainly L1014F mutations (kdr - knock-down resistance) have been identified. Kdr mutations are present in a very large number of populations in the North, West and South of France and confer moderate resistance levels. In rape winter stem weevil (*Ceutorhynchus picitarsis* G.) populations, two main resistance mechanisms have been identified: target gene mutation (L1014F) and resistance by detoxification which is strongly suspected.

Unlike the previous three pests, for *Phyllotreta* sp., rape stem weevil (*Ceutorhynchus napi* G.), cabbage stem weevil (*Ceutorhynchus pallidactylus* M.) and the cabbage seedpod weevil (*Ceutorhynchus assimilis* P.) no return of loss of efficiency is observed in the field. However, the first pyrethroid mutations have been detected for some of them [2]. To maintain the effectiveness of the insecticides and provide appropriate treatment advice, it's necessary to continue the monitoring of these species.

* French plant health association

Keywords: oilseed rape, coleoptera, resistance, pyrethroids, *Psylliodes chrysocephala*, *Ceutorhynchus picitarsis*.

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[1] Robert C. et al, 2019. Coléoptères ravageurs du colza : résistances aux pyréthrinoïdes, *Phytoma*, 724, 20 -24.

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S1-2: Parasitoid's vertical distribution in flowering oilseed rape and implications for insecticide application

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Parasitoids are a key element of integrated pest management in oilseed rape. Particularly important are larval parasitoids. According to the life cycle of their host larvae, many parasitoid species are abundant in the crop during the period of flowering. Insecticides applied against pod damaging insect pests can have detrimental effects on beneficial organisms such as parasitoids. To avoid this, farmers are encouraged to apply damage thresholds, use selective insecticides and apply insecticides temporally targeted (e.g. only in the evening for bee protection). With the so-called dropleg technique, pesticides can also be applied spatially targeted below the canopy of open flowers and pods. The technique was shown to reduce pesticide residues in nectar and pollen after applications in flowering oilseed rape.

To evaluate whether spatially targeted applications of insecticides using dropleg technique may benefit parasitoid species, in a first step the vertical distribution of parasitoids in flowering oilseed rape was studied during two years. Additionally, field trials using conventional and dropleg application techniques were conducted and effects on important parasitoids of the pollen beetle and the cabbage seedpod weevil were assessed. A portable suction trap was used to sample parasitoids from different crop layers during the flowering periods in 2018 and 2019. Results indicate a species-specific preference of either the upper crop canopy including open flowers, pods and buds or the below flowering canopy including stems and leaves. For the most abundant pollen beetle parasitoid in central Germany, *Tersilochus heterocerus*, field trials proved dropleg application technique to reduce negative effects of insecticides on larval parasitism rates. Instead, larval parasitoids of the cabbage seedpod weevil were not influenced by the type of application technique. In general, the temporal coincidence between parasitoid migration into the crop and insecticide application determined the extent of side effects.

Keywords: dropleg technique, pollen beetle, cabbage seedpod weevil

References:

[1] Hausmann, J. et al, 2021. The effect of insecticide application by dropleg sprayers on pollen beetle parasitism in oilseed rape. *BioControl* vol. 66, p. 765-777.

S1-3: Prospects for research on RNAi-based protection of oilseed rape

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Given the large amount of Europe's land that is used to grow oilseed rape (*Brassica napus*), and the detrimental effects that conventional crop protection measures (e.g. broad-spectrum pesticides) can confer to non-target organisms, ecosystem services and crop productivity, the development of sustainable pesticides is gravely needed. RNA interference (RNAi) applications are gaining traction as viable measures for managing crop pests. The benefits of this biotechnology lie in the associated biosafety to non-target organisms and surrounding environments. In brief, this biosafety is due to the nucleotide sequence-specific mode of action of double-stranded RNA (dsRNA, the active pesticide compound); dsRNA is a natural polymer that, when processed within the cell, can reliably result in targeted downregulation of specific genes in a given species of interest.

Here we discuss the prospects for advancing research towards RNAi-based protection of oilseed rape; we assess the complexities, unknowns and obstacles to achieving headway in this sector. Focusing on several key oilseed rape pests in Europe, we consider RNAi-related knowledge gaps in pest physiology, pest niche and other ecological aspects of potential interest for RNAi-based approaches and steps involved in the progression from bioinformatics to lab, semi-field and field.

Keywords: RNA interference, double-stranded RNA, biopesticide, spray-induced gene silencing, transgenic, rapeseed, beetle, Coleoptera, pathogens, Europe

S1-4: Laboratory assessments of the potential of biopesticides to control the cabbage stem flea beetle

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The cabbage stem flea beetle (CSFB) is an economically important pest of oilseed rape in the UK [1][2]. This pest was effectively controlled by neonicotinoid insecticide seed treatments until the active ingredients used for this purpose were banned by the European Union in December 2013 [3]. Since then, CSFB have been difficult to control as populations in the UK developed high levels of resistance to pyrethroid insecticides [4], the only authorized product to control this pest. Alternative solutions are necessary, and one component of future Integrated Pest Management (IPM) programmes for this pest may be the use of biopesticides.

In the laboratory, we tested entomopathogens such as entomopathogenic fungi, and bacteria; physically acting products such as fatty acids; and as a positive control, the pyrethroid lambda-cyhalothrin, against CSFB adults, either sprayed directly on the pest (fungi, physically acting products and pyrethroid) or given to the pest through treated food (bacteria).

Fatty acids were effective with more than 85% of CSFB dead 24 hours after treatment. The entomopathogenic fungus *Beauveria bassiana* (Botanigard WP) led to 56% mortality 14 days after treatment when applied at double the field rate. Entomopathogenic bacteria formulations were not effective, mortality remaining below 50% and not significantly different from the control. Foliar applications of lambda-cyhalothrin under laboratory conditions resulted in 100% CSFB mortality when the field rate was used. We conclude that entomopathogenic fungi and fatty acids could potentially be used as control agents of CSFB and will be investigated further in the laboratory and in the field.

Keywords: Cabbage stem flea beetle, biopesticides, entomopathogens, biological control, entomopathogenic fungi

References:

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S2-1: Perspectives for integrated insect pest protection in oilseed rape breeding

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In the past, breeding for incorporation of insect pest resistance or tolerance into cultivars for use in integrated pest management schemes in oilseed rape/canola (*Brassica napus*) production has hardly ever been approached. This has been largely due to the broad availability of insecticides and the complexity of dealing with high-throughput phenotyping of insect performance and plant damage parameters. However, recent changes in the political framework in many countries demand future sustainable crop protection, which makes breeding approaches for crop protection as a measure for pest insect control attractive again. At the same time, new camera-based tracking technologies, new knowledge-based genomic technologies and new scientific insights into the ecology of insect–Brassica interactions are becoming available.

Here we discuss and prioritise promising breeding strategies and direct and indirect breeding targets and their time-perspective for future realisation in integrated insect pest protection of oilseed rape. In conclusion, researchers and oilseed rape breeders can nowadays benefit from an array of new technologies, which in combination will accelerate the development of improved oilseed rape cultivars with multiple insect pest resistances/tolerances in the near future.

Keywords: Oilseed rape, insects, integrated pest management, resistance breeding, high-throughput phenotyping, genomics

References:

Obermeier C., Mason A.S., Meiners T., Petschenka G., Rostas M., Will T., Wittkop B., Austel N., 2022. Perspectives for integrated insect pest protection in oilseed rape breeding. *Theor Appl Genet*, doi: 10.1007/s00122-022-04074-3

S2-2: Evaluation of varietal differences in vigour and tolerance to flea beetle larvae (*Psylliodes chrysocephala*) among rapeseed varieties grown in France

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In recent years, winter oilseed rape (WOSR) production in France have become increasingly challenging due to environmental changes (pest pullulations, climatic hazards), land use and practice evolutions (diminution of insecticide solutions). One of the possibilities to overcome some of the limitations of WOSR production is to identify and assess new suitable varietal traits involved in insect tolerance.

Terres Inovia established a method based on the use of UAV (Unmanned Aerial Vehicle) to estimate a new phenotypical trait for farmers: the winter oilseed rape vigour. We studied the evolution of WOSR soil cover in autumn using RGB (Red Green Blue) UAV photos. We established a methodology that is reliable, robust, repeatable, and more precise than common method based on human observations. We found significant genetic diversity of vigour between the most common cultivated varieties in France. The results of this study allowed the French farmers to access to a new varietal factor to choose the most appropriate variety for their farm.

Differences in tolerance to flea beetle larvae in oilseed rape varieties have been observed in the field. Terres Inovia and its partners have established a network of plots without insecticide treatments to evaluate these varietal differences among varieties grown in France. The institute showed significant differences in the presence of flea beetle larvae in the plant and larval damage in spring, between varieties. A study is underway to create an indicator combining vigour and tolerance to flea beetle larvae to provide the best advice to rapeseed producers.

Keywords: oilseed rape, *Psylliodes chrysocephala*, varietal vigour, varietal pest tolerance.

References:

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- [2] *Vigueur variétale du colza et comportement face aux ravageurs d'automne - RTTI*, <https://www.youtube.com/watch?v=qbtxDyvnG4c>, 2021.

S2-3: Phenotyping of *Brassica juncea-fruticulosa* introgression lines for resistance against *Lipaphis erysimi* (Kaltenbach)

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Turnip aphid, *Lipaphis erysimi* (Kaltenbach) is an important pest of oilseed *Brassica* in Indian subcontinent. Our group at Ludhiana has introgressed gene(s) for aphid resistance from *Brassica fruticulosa* to *B. juncea*, among which three were found resistant to this pest but with some linkage drag. Thus, these lines were further subjected to γ irradiation to optimize the introgressed segment. Among 159 γ -irradiated introgression lines phenotyped for two consecutive years (2019-20 and 2020-21) under field conditions, four (P64-9, P64-10, P64-39, P64-49) were found to be resistant consistently while 39 were moderately resistant, 28 susceptible whereas 76 were highly susceptible. The promising introgression lines under field conditions were further evaluated under laboratory conditions which revealed significant reduction in nymphal survival, fecundity and adult longevity as compared to check. However, nymphal development period increased significantly in resistant genotypes. Based on the pooled data of both field and laboratory study, 26 genotypes were found to be promising with respect to different parameters studied. These resistant genotypes can serve as an important source of resistance in breeding for aphid resistance in oilseed *Brassica*.

Keywords: Aphid, *Brassica*, Introgression lines, Phenotyping, Resistance

S2-4: Of glucosinolates and saponins - Pollen beetles can cope with general but not with specific plant defence compounds in crucifers

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The pollen beetle (*Brassicogethes aeneus*) is one of the major insect pests of oilseed rape (*Brassica napus* L.; *Brassicaceae*) causing significant seed yield losses. Since pollen beetles become increasingly resistant to pyrethroids, alternative control strategies within the framework of integrated pest management are urgently needed. To develop new strategies against the pollen beetle we studied the natural chemical variation in brassicaceous plant species and identified plant compounds mediating reduced feeding damage. Reduced feeding damage has rarely been observed in oilseed rape and *B. napus* resyntheses, but could be demonstrated for *Barbarea vulgaris*, *Eruca sativa* and *Sinapis alba* [1]. The beetles' sex and the plant accession affected the feeding response of the pollen beetle to these three species.

To identify feeding deterrent secondary plant compounds, we compared semi-polar metabolite fraction profiles of *B. vulgaris*, *E. sativa*, *S. alba* and *B. napus* by a non-targeted approach. Here we selected the discriminating features showing negative correlation with the beetles' feeding behaviour and identified several glucosinolates, flavonoids and saponins [1]. We tested the direct influence of single metabolites achieved as commercial standards or from fractionated plant extracts in a newly designed dual-bud-choice assay. Whereas we observed no effects for the flavonoids, all five saponins from *B. vulgaris* deterred the beetles from feeding. From the six tested glucosinolates only the *B. vulgaris*-specific glucobarbarin deterred the beetles from feeding.

We conclude that pollen beetles are well adapted to defence compounds from a wide range of cruciferous species, but not to specific compounds from *B. vulgaris*. Furthermore, we highly recommend securing correlations between metabolites and feeding behaviour by performing dose response experiments with single putative marker compounds to unravel the chemical signals mediating plant-insect interaction in crop plants.

Keywords: secondary plant compounds, plant-insect interaction, metabolome profiling

References:

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S2-5: Chemical bases of host-plant rejection in a pollinivorous insect facing flower buds

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Host-plant selection by phytophagous insects highly depends on plant chemistry, from volatiles influencing distance recognition to surface and internal metabolites for contact recognition. Metabolites acting at contact have a direct impact on the feeding behavior, and include both stimulant compounds such as sugars and deterrent compounds such as defense compounds. The balance between these stimulant and deterrent metabolites finally determines the host-plant range of the insect, and why certain plants are accepted while others are rejected.

In the present study, we try to understand the chemical bases of host plant rejection in a pollinivorous insect, the pollen beetle (*Brassicogethes aeneus*). Although generalist, the pollen beetle feeds mainly on brassicaceous plants, on which it shows both inter and intraspecific feeding preferences when flower buds are still closed and must be pierced to access the pollen. In particular, we showed that the white mustard (*Sinapis alba*) is less accepted than oilseed rape (*Brassica napus*) and that variation exist between mustard populations. In parallel, previous studies have suggested that the chemical composition of the perianth, the outermost tissue of flower buds, could be correlated with feeding intensity [1].

To investigate the chemical basis of the rejection of the white mustard, a bioguided fractionation approach has been developed: perianth compounds were extracted and fractionated, and then tested on the pollen beetle in a series of feeding tests performed on artificial substrates. Our results confirm that the feeding contrast observed on entire plants is partly related to the phagodeterrent effect of compounds present in the perianth. An additive or synergistic action of several semi-polar compounds seems to be at the origin of this effect. We further explored the effect of compounds present in anthers and found that they may also participate to the rejection process. Untargeted metabolomics will follow to identify perianth and anther compounds, and to test their effect on the pollen beetle.

Keywords: plant-insect interactions, host-plant selection, chemical ecology, specialized metabolites, macronutrient content

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S3-1: Understanding how oilseed rape crops are colonised by one of its main pests, the cabbage stem flea beetle *Psylliodes chrysocephala*

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Integrated pest management is an emerging solution to protect crops using less or no insecticides. However, predicting crop colonisation and the pest's spatial distribution is essential for effective control. The cabbage stem flea beetle *Psylliodes chrysocephala* (CSFB) can cause complete destruction of oilseed rape crops (*Brassica napus*, OSR).

In the present study, the colonisation process of OSR fields by the CSFB was studied using sticky traps placed at the border and in the centre of the fields at different heights. Results show that more individuals were caught on the outer side of the traps, suggesting that more beetles were entering than leaving the crop, and beetles were moving into the centre quickly. Catches were more important at the bottom of the trap near the crop, and they were more important during the day than during the night. The sex-ratio of individuals caught was both equilibrated and stable over the study period. Dissections of female's genitalia showed that sexual maturity occurred during the experimentation. Finally, the integration of sampling data with local meteorological data showed that the caught depends on temperature, humidity, and wind speed. The information provided by this study could contribute to implement IPM strategies against this pest.

Keywords: cabbage stem flea beetle, oilseed rape crops, integrated pest management, crop colonisation

S3-2: Regular and synchronous population cycles of cabbage stem flea beetle facilitate integrated pest management

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Cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala*) is a serious insect pest in winter oilseed rape in Europe. The beetle has become increasingly difficult to manage following insecticide resistance development and restrictions on the use of insecticides. We analysed data on CSFB larval densities in 3045 winter oilseed rape fields in Scania, southernmost Sweden that has been collected over half a century (1969-) with the original aim to predict the need of insecticide seed treatment for the following growing season.

We found that CSFB exhibits strong 8-year population cycles that were stable over the period and largely synchronous across five subareas within the region, although stronger in the southwest compared with the northeast. Later sowing, fewer larval accumulated degree days and number of days with strong frosts with temperatures below -10°C during winter explained additional variation and were all correlated with lower pest density. Intraspecific competition or predator-prey dynamics are potential drivers of this cyclicity and synchronicity, whereas bottom-up effects through changes in host plant quantity or quality seem unlikely as drivers. Irrespective of mechanisms involved, the cyclic CSFB population dynamics means that pest damage can be predicted long before the crop is sown. Proactive pest management tactics can therefore be implemented and tailored to expected pest pressure, and can include a program for threshold-based use of insecticide seed treatments.

Keywords: *Brassica napus*, monitoring, prognosis, *Psylliodes chrysocephala*, Sweden

S3-3: Insights into the aestivation in cabbage stem flea beetle (*Psylliodes chrysocephala*)

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The development of sustainable management methods depends on the thorough understanding of the biology of the target species. The Cabbage Stem Flea Beetle (CSFB), *Psylliodes chrysocephala*, is the most prominent pest of oilseed rape in Europe [1], but little is known regarding its summer diapause (aestivation). The aestivation response in CSFB is clearly a critical stage in its life cycle that enables the adults to survive the summer and recolonize oilseed rape fields in autumn [2].

The main goal of our experiments was to reveal insights into the physiological/behavioral alterations occurring in aestivating CSFB adults. We used a population, which has been reared in controlled environmental conditions (21°C, 16:8 light to darkness) for several years, and for which a shortened diapause is suspected. Specifically, we investigated oviposition, gonad morphology, CO₂ emission, and feeding activity in CSFB males and females from eclosion onwards until aestivation was completed.

The CO₂ emission rates were recorded with a CO₂ analyzer at 5-day intervals. The feeding activity was determined by measuring the leaf area consumed daily, using imaging software. Oviposition behavior was assessed daily and these data was juxtaposed with pictures of dissected gonads, indicating maturity.

The CO₂ emission recordings revealed a major reduction in the metabolism of beetles of both sexes' beetles during the first two weeks after eclosion. Monitoring of the feeding activity revealed a clear feeding pause between 13 and 35 days after eclosion. Oviposition did not begin until 48 days after eclosion and the median on which females laid their first eggs was 52 days post eclosion. Gonad pictures were in line with this finding as the first matured ovaries were sampled from 40-day-old females. Overall, our results provide evidence that aestivation is associated with a range of behavioral and physiological changes including lack of reproduction, feeding activity, and reduced metabolism.

Keywords: Aestivation, Cabbage stem flea beetle, summer diapause, *Psylliodes chrysocephala*

References:

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S3-4: Ecology and distribution of cabbage stem flea beetle and its parasitoids in UK winter oilseed rape crops: steps towards Integrated Pest Management

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The cabbage stem flea beetle (CSFB, *Psylliodes chrysocephala* L.) is the most abundant and widely distributed autumn pest of winter oilseed rape (OSR, *Brassica napus* L.) [1]. A combination of the neonicotinoid seed treatment restrictions and resistance to pyrethroid insecticides [2]- the only other remaining control option, has left farmers with limited control options resulting in drastic reductions in production[3]. Integrated pest management (IPM) may offer a solution to combat CSFB in a sustainable and efficient way.

The aim of this study was to better understand the ecology of CSFB and develop integrated pest management techniques, in particular the role of beneficial parasitic insects in CSFB mortality. This project involved monitoring the migration and daily activity of CSFB in the field, modelling climate and agricultural management impacts on the spatio-temporal changes in CSFB populations. The management and environmental factors affecting CSFB larval populations were analysed using a combination of statistical methods. Sowing date, field area and the interaction between temperature and rain in September, October and November and mean temperature in December and January had a significant effect on the number of CSFB larvae.

This study has also updated the distribution and parasitism rates of CSFB larval parasitoids in the UK and optimized DNA metabarcoding methods to identify larval parasitoids and estimate parasitism levels. For the first time, this research has studied the spatiotemporal distribution and biocontrol potential of *Microctonus brassicae*, the main parasitoid attacking adult CSFB. It has shown that *M. brassicae* is widespread across the UK with parasitism rates ranging from 0 to 36%.

Keywords: biocontrol, *Brassica napus*, *Psylliodes chrysocephala*, insect pest control, pesticides, sustainable agriculture

References:

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S3-5: Assess cabbage stem flea beetle (*Psylliodes chrysocephala* L.) and rape winter stem weevil (*Ceutorhynchus picitarsis* G.) risk by integrating an agronomic and a pest risk.

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The cabbage stem flea beetle (*Psylliodes chrysocephala* L.) and the rape winter stem weevil (*Ceutorhynchus picitarsis* G.) have developed several pyrethroid resistance mechanisms since 2014-2015 [1]. Next autumn, there will have no more available insecticide alternative to pyrethroids.

More than ever, the combination of agronomic levers (early sowing, nitrogen fertilization strategies, association with frost sensitive legume crop) is essential to limit the attacks of these pests [2] [3] and their harmfulness and maintain the sustainability of pyrethroids in areas where they are still effective. Studies carried out for several years have demonstrated the importance of plants with an important biomass [2] [3], a continuous growth throughout the autumn and a rapid recovery in spring to reduce the harmfulness of these two pests.

To estimate the risk of yield losses as accurately as possible, it is necessary to integrate not only a pest pressure indicator but also indicators to assess the growth dynamics of oilseed rape in autumn and in early spring. Terres Inovia, using its expertise, built decision rules, available online, combining indicators of pest pressure and oilseed rape growth [4] [5].

Keywords: *Psylliodes chrysocephala*, *Ceutorhynchus picitarsis*, risk evaluation, agronomy.

References:

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- [5] <https://www.terresinovia.fr/-/charancon-bourgeon-colza>

S3-6: Control of *Ceutorhynchus napi* and *C. Pallidactylus*

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Ceutorhynchus napi and *C. pallidactylus* appear in winter oilseed rape in early spring. Depending on the temperature, the females of *C. napi* need only a few days of maturation feeding before they start oviposition. With their oviposition, the damage is initiated and stems may become s-shaped or can burst open. In addition, the larvae damage the plants by their feeding by disturbing the transport of water and nutrients within the stems. During flowering, the larvae complete their development and leave the plants to pupate in the soil, where they stay until the next spring. Compared to *C. napi*, females of *C. pallidactylus* need some more time before they start with oviposition (depending on temperature approximately 10-14 days). The larvae also feed within the stems. After finishing development, larvae drop down the plants to pupate in the soil. In contrast to *C. napi*, the next generation of *C. pallidactylus* emerges already in June/July from the soil and searches for their overwintering sites in the upper soil layer and leaf litter in edges of forests and hedges. Immigration of both weevil species can be monitored in spring with yellow water traps (YWT). After exceeding the thresholds (>5 *C. napi* / >15 *C. pallidactylus* per YWT within three days), the only control option in Germany are insecticides from the class of pyrethroids. While *C. napi* is still sensitive against this insecticide class, *C. pallidactylus* shows a beginning resistance to pyrethroids. For an effective control, the timing of insecticide application is very important. This requires a better knowledge of the biology of the weevils. Results from perennial field trials near Braunschweig will be reported.

Keywords: rape stem weevil, cabbage stem weevil, oviposition, larvae, timing of insecticide control

S4-1: Combining agronomy and conservation biological control at a territory scale for pest management

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Since 2009 in France and notably in Burgundy, pest control in oilseed rape has become tricky. Indeed, cabbage stem flea beetle (*Psylliodes chrysocephala*) and rape winter stem weevil (*Ceutorhynchus picipitarsis*), two major pests in WOSR have become resistant to pyrethroids. Farmers implementing short rotations and particularly those with weak soil potential are facing economic and technical issues.

“R2D2” territory project, settled for 6 years on a 1300 hectares field crops area, aims at helping ten farmers to manage pests without insecticides combining agronomy and conservation biological control to increase crop robustness and pest regulation by their natural enemies. Farmers are supported to design and implement step by step a territorial project with low dependence on insecticides, in an adaptive management approach. It is based on individual and collective technical support, training, co-design workshops, and on-farm generative experimentations. Flower strips and hedgerows plantations, push and pull strategies at territory scale and intercropping are examples of collective actions designed and settled at a landscape scale.

Keywords: agroecology, pest management, conservation biological control, oilseed rape, landscape management, agronomy, cropping systems

S4-2: When intercropping also rhymes with insect pest management: the case study of oilseed rape

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Intercropping is notoriously known to manage weeds in oilseed rape (*Brassica napus*), but what if it would have the additional benefit to protect the cash crop from insect pest attacks? In an field experiment, we have benefited from a mild winter to evaluate the impact of faba bean (*Vicia faba*), an intercropped species which did not freeze, on the insect pest complex during winter and spring as compared to control pure oilseed rape. Insect damage by the beetles *Psylliodes chrysocephala*, *Ceutorhynchus napi*, and *Brassicogethes aeneus* was assessed in both treatments. The larval density of *P. chrysocephala* was significantly lower in the crop grown with service plants. Egg laying and damage by *C. napi* were significantly reduced when oilseed rape was intercropped, and the number of *B. aeneus* captured was significantly lower in the presence of service plants than in the control. Last but not least, we could measure a significant increase in yield in the intercropped plots as compared to the control oilseed rape. We are currently trying understand the mechanisms, but the present results open the door to inovative and sustainable insect pest management practices which have the potential to reduce insecticide applications while maintaining competitive oilseed rape yield.

Keywords: cabbage stem flea beetle, rape stem weevil, pollen beetle, integrated pest management, functional biodiversity

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S4-3: Flower strips do not constitute an aestivation site for cabbage stem flea beetles, in contrast to woodland edges

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Semi-natural habitats, like woods, hedges and flower strips, are generally beneficial to natural enemies, and flower strips increase biological control [1]. However, these habitats can also be used by pests, such as the cabbage stem flea beetle, *Psylliodes chrysocephala* (Linnaeus, 1758, Coleoptera: Chrysomelidae), a major pest of winter oilseed rape, *Brassica napus* L. Adults emerge in late spring and move to aestivation habitats for their summer diapause, looking for shelters where they can hide in the vegetation [3]. According to the literature, the major shelter used is forest edges [2], but flower strips could be suitable too. It is important to know this ecological characteristic in order to manage this insect at the landscape level.

In this study, we had two objectives: (i) determining and comparing the importance of three habitats (flower strips, a former winter oilseed rape field and a north-facing woodland edge) during the aestivation, and (ii) identifying which habitat characteristics are involved in the constitution of a favourable habitat. Therefore, the emergence of stem flea beetles was monitored with emergence traps laid in each habitat from mid-August to mid-October 2021, in 14 landscape sectors containing the three types of habitats studied. In each habitat, the local environment characteristics (e.g., surface of plant cover) and landscape composition in a radius of 2 km were described.

The number of aestivating cabbage stem flea beetles was the highest in woodland edges compared to flower strips and former oilseed rape fields. However, great variations were observed between sectors, for the same type of habitat. In each habitat, we investigated the relationships between the amount of aestivating stem flea beetles, local environment characteristics and landscape structure. Our preliminary results suggest that some habitat characteristics present in woodland edges are not found in other habitats.

Keywords: Aestivation, *Psylliodes chrysocephala*, *Brassica napus*, semi-natural habitat, local environment characteristics, landscape

References:

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S4-4: Effects of perennial flower strips on the parasitism of oilseed rape pests

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The implementation of habitats to improve functional biodiversity is known to increase natural enemies of pests, but it does not necessarily lead to a significant reduction in pest populations. The performance of flower strips and natural enemies in terms of biological control depends on many factors and we need to understand why some mixtures of plant species perform better than others.

In a field experiment, we compared the effect of eight contrasting floral mixtures comprising native and perennial plant species. In these mixtures, we have varied the proportion, the species and functional diversity of plant species providing resources towards natural enemies. Each flower mixture was sown on a 6 × 45 m strip and replicated on three blocks. In the adjacent oilseed rape crop, at 5 and 20 m from the strip, we evaluated parasitism rates of *Psylliodes chrysocephala*, *Ceutorrhynchus pallidactylus*, *Brassicogethes aeneus*, *Brevicoryne brassicae* and *Dasineura brassicae*. Vegetation surveys were carried out in the spring to describe the vegetation. We used a mechanistic trait-matching approach between plant and parasitoids to determine the amount of nectar available and accessible to parasitoids.

In general, parasitism rates, averaged over all mixtures, were below 1 % for *B. brassicae*. They varied from 11 % for *B. aeneus* to 42 % for *D. brassicae*. When we compared parasitism rates across distances, no general distance effect was detected. At a distance of 5 m from the strips, the percentage of plant cover providing nectar, alone or in interaction with species number, had a significant positive effect on parasitism rates in all species except *D. brassicae* [1]. Plant specific richness and plant functional diversity had a negligible influence relative to the cover of plants providing accessible nectar.

We show that the biological control of several oilseed rape pests can be strengthened by the establishment of perennial flower strips providing food resources available and accessible to parasitoids.

Keywords: conservation biological control, nectar, *Brassicogethes aeneus*, *Dasineura brassicae*, *Psylliodes chrysocephala*

References:

[1] Gardarin A. et al, 'The hump-shaped effect of plant functional diversity on the biological control of a multi-species pest community', *Sci. Reports* 11 (1):21635.

S4-5: Spatiotemporal distance between oilseed rape fields reduces pollen beetle abundance without affecting biocontrol

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Large monocrops provide sufficient vital resources for agricultural pests, but often do not support the presence of natural enemy populations (e.g. predators, parasitoids) [1–3]. This ecological imbalance can make intensive pesticide use a necessity for farmers [4]. At the farm level, preventive methods in pest control (e.g. landscape complexity, crop-rotation, pathogen-free propagation material, cautious pesticide use) are well-established, whereas spatiotemporal field separation of same crop species remains understudied for most crop–pest systems, yet may be of great importance as a pest management technique.

We carried out a three-year study in winter oilseed rape fields, where half of the fields were within 500 m from the previous year’s closest oilseed rape field, and half outside this spatial distance. In the study, we collected data of pollen beetle (*Brassicoglyphus aeneus*) by using the plant tapping method. Using funnel traps, we collected pollen beetle larvae dropping to soil for pupation to determine the parasitism rate, and yellow water traps were used to observe the flight activity of pollen beetle and its parasitoid wasps.

Pollen beetle abundance was low in all study years. However, their abundance was smaller in fields located further than 500 m from the previous year’s nearest oilseed rape field compared to fields that were closer than 500 m. The parasitism rate of pollen beetle was over 35% in each study year, which surpasses the required level for effective biocontrol [5]. In 2017, the parasitism rate was significantly higher in fields that were closer than 500 m to the previous year’s nearest oilseed rape field.

We demonstrate that spatiotemporal field separation can be an effective preventive pest control technique at the landscape scale, and can work together with conservation biocontrol, which helps reduce the next year’s pest population. The use of spatiotemporal distancing should be investigated as a key integrated pest management strategy.

Keywords: pollen beetle; agricultural land management; integrated pest management; biological control

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S4-6: Waiver of oilseed rape production in a large area for one year to reduce insect pest pressure in the following season?

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Chemical control of insect pests in oilseed rape production became less and less effective in the last years due to resistance and a lack of new MoA. Chemical control at high pest pressure is often not sufficient anymore. Alternative control methods are urgently needed. Theoretically, a waiver of growing oilseed rape in a large area could reduce pest pressure in this area in the following year.

A monitoring was carried out in two growing seasons in regions of about 1000 km² with no oilseed rape production, because of too dry sowing conditions in autumn 2019 and again 2020. Insect pest numbers were recorded on oilseed rape fields in the area with no oilseed rape in the previous season and on fields in the adjacent area with continuous oilseed rape production. Monitored fields were located from 0 up to 10 km away from previous year oilseed rape fields. The results of the first season are less reliable because sowing and emergence of the crop was very variable and several fields had to be abandoned because of crop failure. Taking both seasons together, damage of *Psylliodes chrysocephala* appeared later in the season and to a lower extent and larval numbers tended to be lower, if fields were more far away from previous crops. In addition, *Ceutorhynchus napi* was clearly reduced with larger distances from previous oilseed rape. No effects were visible for *C. pallidactylus* appearing in very high numbers. Pest pressure of *Brassicogethes aeneus*, *C. assimilis* and *Dasineura brassicae* were too low to allow reliable conclusion.

Keywords: Alternative insect pest control, distance, pest insect mobility

S5-1: Identification of the predators of oilseed rape pests and quantification of predation services using camera trapping

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Cameras are becoming common tools for ecologists. They are for example used to record species abundance, richness, and interactions. Cameras are mainly used to study large animals such as mammals, but recently more attention has been paid to invertebrates, to record insect-flower and predator-prey interactions. The importance of predation in biocontrol services for spring-active pests of oilseed rape have been quantified and seems important but variable [1]. The identity of the predators providing this service have been mainly estimated by spatio-temporal correlations between pests and predator species abundance, or by feeding trials conducted in the laboratory. These two approaches are informative but can be strongly biased. Cameras offer an opportunity to identify the predators of a pest with less bias than the standard methods.

Here we present preliminary results from an experiment conducted in the UK where larvae of two of the main oilseed rape pests in spring, pollen beetles and brassica pod midges, were placed on the ground and tracked using camera traps. The experiment was conducted over 14 occasions between June and July. Multiple predation events were recorded and predators were identified, showing that this method can produce useful and reliable results. It was also possible to estimate the time of the day when the predators were more active and identify the time window of susceptibility of the larvae to predation. The potential technical development of this method and the future questions that can be investigated will be discussed.

Keywords: pollen beetle, Brassica pod midge, larvae, camera traps, predation, spring pests

References:

[1] Williams, I. H. (Ed.), 2010. *Biocontrol-based integrated management of oilseed rape pests* (Vol. 461). Dordrecht: Springer.

S5-2: Dynamics of pollen beetle (*Brassicogethes aeneus*) immigration in European oilseed rape

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Pollen beetles are a significant economic pest of European oilseed rape. A better understanding of the spatial and temporal dynamics of *B. aeneus* immigration would enable precise monitoring and management strategies. We used green and yellow water traps, sticky cards, and near-infrared insect sensors to monitor the immigration and dispersion of pollen beetles in an oilseed rape field.

This work provides evidence of pollen beetles aggregation in an oilseed rape field. Early temporary aggregation occurs upwind, while the eventual aggregation appears to occur in the middle or edge of the field. Aggregation occurs prior to OSR flowering.

This work provides further data in support of pollen beetles entering fields upwind. Due to pollen beetle aggregation, a more thorough sampling strategy for the early detection of pollen beetles is recommended, including sampling upwind, if there is a primary wind direction. Furthermore, sensors provide early detection and may be able to be used to set economic thresholds for management in the future.

Keywords: pollen beetle, near-infrared sensor, aggregation, immigration, monitoring

POSTERS

P-1: Incidence of resistance to pyrethroids and carbamates in Czech populations of green peach aphid (*Myzus persicae* Sulz.) from oilseed rape fields

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Since the half of the 20th century, *M. persicae* has developed resistance to wide range of insecticides, including organophosphates, carbamates, pyrethroids, and neonicotinoids. Failures in control of *M. persicae* by pyrethroids and carbamates has been observed in the Czech Republic since 2018. The aim of the study was evaluate the susceptibility of *M. persicae* to various insecticides in the bioassay and prove the presence of kdr mutation in pyrethroid-resistant samples from the bioassay.

Susceptibility of eleven local populations of *M. persicae* collected in Czech oilseed rape fields in 2019-2021 to 13 insecticides from various groups was tested in the IRAC no. 019 and the presence of kdr (L1014F) mutation connected with pyrethroid resistance in *M. persicae* was proved by qPCR in samples from the bioassay survived the treatment with alpha-cypermethrin.

The tested populations of *M. persicae* were highly susceptible to chlorpyrifos-methyl and acetamiprid. Resistance to alpha-cypermethrin and pirimicarb was proved in most of the tested populations. Mortality of *M. persicae* ranged between 7.4% and 98.4% and between 10.7% and 97.4% after application of alpha-cypermethrin and pirimicarb in the 100% of the field application dose, respectively. Sulfoxaflor, flonicamid and spirotetramate, were highly effective in control of *M. persicae* populations resistant to pyrethroids and carbamates. The L1014F mutation was found in all the populations that survived the application of alpha-cypermethrin. The heterozygous (SR) genotype was detected in 46.4% of individuals and 20.0% of the individuals that survived the 100% and 500% of the field application dose of alpha-cypermethrin, respectively. The resistant homozygous (RR) genotype was detected in 20.0% of the individuals that survived the 500% of the field application dose of alpha-cypermethrin. High resistance of *M. persicae* in contrast with the low incidence of kdr mutation indicate the evidence of another mechanism of resistance.

Keywords: green peach aphid; carbamates; pyrethroids; resistance; kdr mutation

P-2: Sensitivity of cabbage flea beetles (Coleoptera: Chrysomelidae) to insecticides

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Cabbage flea beetles (*Phyllotreta* sp.) are very important rapeseed pests in the germination period. Feeding causes round holes in the leaves, that increase in size as leaf surface area increases, and leaf margins necrotize. Young plants are stunted in growth and produce lower yields. The main control measure is chemical control with insecticides. In Hong Kong and Japan, flea beetles have been found to be resistant to certain insecticides[1].

The objective of this study was to test cabbage flea beetle populations for active ingredients from the pyrethroid, organophosphorus insecticide, and neonicotinoid groups.

During the study period (2019, 2020, and 2021), sensitivity testing was conducted using IRAC test methods 011 for pyrethroids, 021 for neonicotinoids, 025 for organophosphorus insecticides[2], and the protocol of Heimbach et al.[3].

Adult forms of cabbage flea beetles were collected from untreated crops (at 30 sites) and delivered to the laboratory. Solutions based on the active ingredients dimethoate, alphacypermethrin, cypermethrin, deltamethrin, and thiacloprid were prepared at 100% and 20% dosages. The prepared solutions were transferred into glass vials, which were placed on a roller. After the vials were dried, ten adult cabbage flea beetles were placed in each vial. The experiment was evaluated after 24 hours and individuals that showed no coordinated movements for 60 seconds were considered dead.

In 2019, 86% of the tested populations were sensitive to dimethoate, while 79% of the tested populations were sensitive to the active ingredient alphacypermethrin and only 14% of the populations were sensitive to the active ingredient thiacloprid. In 2020, all populations tested were sensitive to the active ingredients cypermethrin and deltamethrin. In 2021, 64% of the populations tested were sensitive to the active ingredient cypermethrin and all cabbage flea beetle populations were sensitive to the active ingredient deltamethrin.

Keywords: cabbage flea beetles, oilseed rape, insecticides, resistance

References:

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[3] Heimbach, U. et al., 2006. First steps to analyse pyrethroid resistance of different oilseed rape pests in Germany, *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* 58: 1-5.

P-3: The impact of insecticides applications on the mortality of pollen beetle larvae in winter oilseed rape buds (*Brassica napus* L.)

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In 2021 was carried out the trial that focused on the degree of parasitization of pollen beetle larvae. In the study were 17 insecticide treatments in four replicates were tested. Twenty inflorescences oilseed were collected from each plot. Inflorescences were immediately frozen after sampling from plots (25 m²/one plot) and later the pollen beetle larvae were analyzed in the laboratory. Assessments were the number of flowers per inflorescence, the number of larvae in each flower, the number of flowers damaged by larvae and the number of larvae in inflorescence (analyzed 16,000 specimens). The number of parasitoid larvae and the number of eggs in larval individuals were determined by dissection with usage a binocular microscope. The assessments in field plots were repeated 3 days, 5 days, 7 days after the application of insecticides.

The results of this trial demonstrate the efficacy of insecticides on pollen beetle larvae and their impacts on parasitization. Mostly, the registered doses of pyrethroids were used (lambda-cyhalothrin, gamma-cyhalothrin, cypermethrin, alpha-cypermethrin, zeta-cypermethrin, deltamethrin, tau-fluvalinate, etofenprox, esfenvalerate, indoxacarb), two neonicotinoids (thiacloprid, acetamiprid) and other products considered to be ecologically (spinetoram, spinosad, azadirachtin and orange oil). Monitoring with yellow water traps during the whole season showed the most frequent parasitoid of the pollen beetle is *Tersilochus heterocerus* (Tersilochinae, Hymenoptera) around city Šumperk, Czechia (parasitization 25-98%). Neonicotinoids with systemic efficacy significantly reduced the number of larvae and at the same time reduced the level of its parasitization. However, a „ecological produkt” - "Radiant" (spinetoram), has no negative effect on the parasitization and reduce the number of larvae too, showed a potential to decrease an abundance of pollen beetles in next season. Etheric pyrethroids did not reduce the number of larvae in the flowers at all.

Keywords: pollen beetle, oilseed rape, insecticides, *Brassicogethes*, oilseed pests, parasitoids

P-4: Assessing the effect of six different essential oils on cabbage seedpod weevil (*Ceutorhynchus obstrictus*) and parasitoid *Nasonia vitipennis*

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The Farm to Fork Strategy, the core of the European Green Deal, aims to make food production fair, sustainable, healthy, and environmentally friendly. To reach the goal, we need to find alternative and environmentally safe plant protection products. The cabbage seedpod weevil (*Ceutorhynchus obstrictus* Marsham) is mainly controlled by synthetic insecticides that are harmful to beneficial insects [1–3]. Plant-based essential oils have shown to be effective in controlling several agricultural pests [4,5], but they can also be toxic to beneficial insects. Thus, there is need for thorough testing before using in an agricultural field.

We tested six different essential oils, in four concentrations, both on cabbage seedpod weevil and hymenopteran parasitoid. For this study *Nasonia vitipennis* Walker was used as a model organism to imitate *C. obstrictus* main parasitoids that belong to the family Pteromalidae. We studied the mortality and mobility of *C. obstrictus* after 3 and 24 hours of indirect exposure via treated oilseed rape leaves and flowers but also filter paper.

Cinnamomum verum, at a concentration of 1.5%, caused 82.5% (± 6.77) mortality of *C. obstrictus* after 24 hours that was significantly higher than other essential oil treatments and even exceeded the mortality rate caused by lambda-cyhalothrin used as positive control. No significantly increased mortality of cabbage seedpod weevils were found on *Thymus vulgaris*, *Carum carvi*, and *Cannabis sativa* treatments at the concentration 1.5% after 24 hours, compared to control (Tween80 and acetone). All essential oils caused nearly 100% mortality of *N. vitipennis* already at three times lower concentration (0.5%). The mortality of *N. vitipennis* was under 30% on treatments with 0.1% solution.

We demonstrate that *C. verum* essential oils can be effective to control the cabbage seedpod weevil and represent an alternative to synthetically produced products. However, the same concentration was also lethal to biocontrol agents.

Keywords: weevil; parasitoid wasps; biocontrol; plant-based products; oilseed rape

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P-5: Relative susceptibility of canola and non-canola cultivars to *Lipaphis erysimi* (Kaltenbach)

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Rapeseed-mustard is an important group of winter season oilseed crops. Though, canola cultivars are widely grown in western countries, they are gaining popularity in India with major area under non-canola cultivars. Since, canola cultivars are low in seed glucosinolates, there is general perception among the growers that reduction in glucosinolates will increase their susceptibility to turnip aphid, *Lipaphis erysimi* (Kaltenbach) – a key pest on oilseed *Brassica* in Indian subcontinent. In this study, we have attempted to analyze the differences in susceptibility of canola and non-canola cultivars to this pest and results will be presented.

Keywords: aphid, brassica, glucosinolates

P-6: Developing oilseed rape resistance to feeding by *Psylliodes chrysocephala*: bioassay and considerations.

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Insect pests are the biggest challenge for the crop protection of oilseed rape (OSR) with cabbage stem flea beetle (CSFB), *Psylliodes chrysocephala*, reported as the most important pest of winter OSR [1]. Adult beetles feed on the cotyledons and early-formed leaves at emergence and can threaten crop establishment, however no resistant OSR cultivars are available [2].

Here, we present a novel laboratory method for phenotyping of resistance against feeding of CSFB adults and discuss important aspects to consider. A special seedling bioassay was developed, to enable phenotyping of this highly vulnerable plant stage. Individual seedlings are grown in plastic cylinders (50 ml) in a soil substrate, which allows for a space- and time-saving cultivation. At the cotyledone stage (BBCH 10), each seedling is offered to one adult in a clear plastic cup (500 ml). After 48h, the cotyledons are photographed and the damage assessment is done by measuring the leaf area consumed using imaging software. Adult-stage CSFB for phenotyping bioassays can be obtained in several ways. The easiest option is to collect them at harvest or from the OSR crop after immigration, but this allows phenotyping only for a short period. The availability of adult beetles can be extended by obtaining larvae from field-collected plants in winter and rearing them to adulthood in controlled conditions. Permanent rearing enables availability of adults and larvae throughout the whole year. If either of the two latter origins is chosen, it has to be considered that CSFB adults undergo a period of summer diapause, which may influence their host plant selectivity.

Our results show that the presented bioassay is a reliable and efficient method for detecting differences between *Brassica* accessions regarding their resistance to CSFB adult feeding. Detailed knowledge on the development phases of different populations is crucial to ensure that the beetles are in an authentic physiological stage when used for phenotyping.

Keywords: Cabbage stem flea beetle, resistance screening, bioassay, seedling, aestivation, phenotyping

References:

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P-7: Identification of a palatable white mustard variety to cabbage stem flea beetle feeding

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Currently, no cabbage stem flea beetle resistant varieties of *Brassica napus* are commercially available. Identification of resistance genes is hampered by challenges with typical phenotyping methodologies using field trials, due to unpredictable infestation rates and difficulty identifying nuanced resistance. In addition, no published rearing methods for this non-model insect currently exist for experimentation in a controlled laboratory setting.

This work optimised methodology for rearing the cabbage stem flea beetle to obtain a consistent source of insects. In addition, a novel phenotyping methodology was used to screen for resistance in the laboratory. Accessions of both *Brassica napus* and *Sinapis alba* were phenotyped, and two genotypes of *Sinapis alba* with contrasting antixenotic (deterrent) resistance were identified. Investigation of quantitative trait loci involved in resistance is underway, with the aim of implicating resistance genes.

Keywords: Cabbage stem flea beetle, resistance, breeding, white mustard

P-8: Monitoring of a cabbage stem flea beetle (*Psylliodes chrysocephala*) population on an oilseed rape field during winter 2021/2022

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Different methods were used on an oilseed rape field to monitor the population of *Psylliodes chrysocephala* (Yellow water traps, direct observations at light and dark conditions and eelectors) between September 2021 and March 2022 on one field in Braunschweig. In Yellow water traps beetles were mainly caught until mid of November, whereas in direct observations the number of beetles was reduced only after begin of January. Clearly higher beetle numbers were seen in the dark compared to dawn or daylight. Number of beetles seen was similar at air temperatures between 0 and 20°C. Beetles could be easily seen in the dark using a torch until leaves of plants began to overlap. Beetle numbers differed clearly between two monitoring areas on the field with only 50 m distance for the whole observation period. Numbers of beetle per m² were similar comparing direct observations and using eelectors.

Keywords: counting method, activity period, direct observation, yellow water trap

P-9: Effect of leaf area loss and introduction of *Psylliodes chrysocephala* larvae on Oilseed rape (*Brassica napus*) growth and yield.

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In two experiments, the ability of OSR to compensate for injury caused by controlled levels of adult and larval stages of *P. chrysocephala* was assessed. Leaf area was removed from young plants to simulate differing intensities of adult feeding injury alone or in combination with manipulation of larval infestation levels. Resulting OSR growth and production in terms of seed quantity and quality was assessed. Both Experiments showed that leaf area loss at early growth stages of OSR can be compensated for, with no impacts on yield. There was no significant effect of larval infestations of five or less larvae (the current UK threshold at which treatment is recommended) but significant impacts were observed from OSR infested with more than five larvae. The data suggest that the current application thresholds for both adult and larval stages of *P. chrysocephala* which trigger insecticide application are below the physiological injury level which plants can fully compensate for damage. This disparity may be leading to higher or earlier application of insecticide that is not necessary to protect yield and this may in turn exacerbate the development and spread of insecticide resistance in *P. chrysocephala*. Implications for application thresholds are discussed.

Keywords: Oilseed rape, cabbage stem flea beetle larvae, crop growth, yield.

P-10: Long-term trends in *Brassicogethes aeneus* abundance in the UK.

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The pollen beetle (*Brassicogethes (Meligethes) aeneus*) is a pest that can cause yield loss in oilseed rape (*Brassica napus*) through feeding on the unopened buds and spoilage in horticultural crops. Outbreaks occur most years, however the timing and abundance of the pest varies from year to year meaning that management is not always necessary to protect crops. Understanding what drives pollen beetle abundance may improve decision support systems and reduce prophylactic use of chemical control. To improve understanding, data on pollen beetle numbers caught in two Rothamsted suction-traps in south-east England between 1987 and 2018 were investigated to determine long-term trends in annual counts and their relationship with area of oilseed rape grown at a national and regional level. The trend at seasonal and monthly levels were compared to the overall trend, and the relationship between counts in late summer of newly-emerged beetles and counts in spring/early summer of overwintered beetles the following year and the inter-generational relationship within year were also investigated.

Keywords: pollen beetle, long-term monitoring, suction-traps.

P-11: The benefits and risks of including Brassicaceous plants in field margins to promote conservation biocontrol of specialist oilseed rape pests

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Loss of biodiversity from arable landscapes has resulted in a deterioration of natural pest control and other ecosystem services, and remediative efforts aim to design field margins that provide multiple services to crops throughout entire rotations. The inclusion of 'banker plants' within these margins to provide an alternative source of host or prey insects has potential to enhance natural enemy populations. Where the enemies of a target pest are specialists, however, banker plants must host pest species, presenting the risk of exacerbating crop infestations.

We assessed the potential of six Brassicaceous plants to enhance parasitoid populations, versus the risk that they might promote those of the pests. Fodder radish *Raphanus sativus* cv. Apoll facilitated high production of parasitoids of the pollen beetle *Brassicogethes aeneus*, but there was strong evidence that this brassica could assist proliferation of the brassica specialist weevil pests *Ceutorhynchus obstrictus* and *C. pallidactylus*. Turnip rape *Brassica rapa* cv. Jupiter, and the *B. rapa* hybrid 'Tyfon' showed potential to perform a trap cropping function, but the early flowering phenology of these brassicas led to a large proportion of the *B. aeneus* larval population escaping parasitisation, potentially assisting proliferation of this pest. Forage rape *B. napus* cv. Hobson exhibited similarly high *B. aeneus* parasitoid production characteristics compared to *R. sativus*, but there was no evidence that this *Brassica* would exacerbate problems with other pests, indicating that it would be a favourable banker plant option.

Our study illustrates that plants capable of promoting parasitoids facilitating biological regulation of one pest can also serve to exacerbate problems associated with a different pest of the same crop. The designers of multi-functional field margins must thus pay as much attention to the negative aspects of the plants they consider for inclusion as they do the positive aspects.

Keywords: Banker plant, biological control, integrated pest management, *Brassica*, pollen beetle

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P-12: Developmental and enzymatic defense response of hemp aphid *Phorodon cannabis* to the selected abiotic stresses

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Phorodon cannabis is an important pest of hemp [1]. Aphids are very sensitive and they developed mechanisms of quick adaptation to changing environmental conditions. The strategies are expressed in their morphology, biology and behaviour [2]. The aim of this study was to find out how the hemp aphid responds to the selected abiotic factors, especially the effect of temperature and pesticide on survival, development, fecundity and demographic parameters. We have measured whether these mild abiotic changes affect the activity of defense response enzymes in aphids, which enables the neutralization of ROS and harmful metabolites.

To investigate the effect of temperature on survival, development, fecundity and demographic parameters of *Phorodon cannabis*, the experiment was carried out at the 4 constant temperatures. The length of the developmental stages and fertility of females was recorded. The variants of the experiment were aphids feeding on hemp plants treated with the herbicide Pantera 040 EC – the selective graminicide containing quizalofop-P-tefuryl (40 g l⁻¹, 4.38%, HRAC group 1: inhibition of Acetyl CoA carboxylase), commonly used to control weeds in hemp.

We studied the biology of hemp aphids and their reaction to temperature and the herbicide. Hemp aphids preferred moderate temperatures between 20°C-25°C. At this temperature range they lived about 25 days out of which they reproduced for 15 days and the average fecundity ranged from 54.5 to 111.6 nymphs per female. At the temperature 28°C the aphids could still thrive, eat and reproduce but the demographic parameters were much lower. Treatment of the crop with the herbicide, caused a mild stress in aphids feeding on treated plants, increased aphid reproduction and changed their feeding habits. The herbicide treatment increased the activity of SOD, CAT, β -glucosidase, GST, POD, and PPO, which indicates a mild stress in aphid tissues, coinciding with higher reproduction and changed feeding behaviour [3]. This study points out the non-target organisms are also affected by pesticide treatments but the effect can be counterproductive.

Keywords: hemp aphid; temperature; herbicide; enzymatic markers; demographic parameters

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P-13: Effect of herbicides and aphids on the synthesis of fatty acids in *Cannabis sativa*

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Cannabis sativa L. is a plant widely used as a source of highly valued, psychoactive and also hallucinogenic medicinal phytocannabinoids, such as cannabidiol (CBD) and for the production of unsaturated fatty acids, with their antioxidant effects valuable for human health [1]. Growing a plant requires the use of crop protection agents such as insecticides, fungicides or herbicides. Crop protection agents prevent the overgrowth of pests or weeds, but on the other hand, they can directly affect *C. sativa*. It has been shown that herbicides containing quizalofop-P-tefuryl can cause disturbances in the synthesis of fatty acids in monocotyledonous plants [2]. The aim of our research was to investigate the effect of the herbicide, containing quizalofop-P-tefuryl and aphids *Phorodon cannabis*, on the synthesis of fatty acids in *C. sativa* leaves.

Oil hemp plants of the Henola (Institute of Natural Fibres and Medicinal Plants, IWNI RZ, Poznan, Poland) variety were deliberately colonized by aphids *P. cannabis*. There were 35 aphids applied to each 6-week old hemp plant. The experiments were carried out in a growth chamber with 16L:8D photoperiod, with day and night temperatures of 23 ± 1 °C and 19 ± 1 °C. Fatty acids were determined as methyl esters after in-situ transesterification according to Wychen et al. [3]. Residues of quizalofop-P-tefuryl were extracted from leaves by acetone: hexane mixture (1:9 v/v). The determination of fatty acids and quizalofop-P-tefuryl was carried out using a gas chromatograph connected to triple quadrupole mass detector. Changes in the level of lipid in plant leaves were also checked using Fourier transform infrared (FTIR) spectroscopy.

Changes in the fatty acid profile in the leaves were observed in response to aphid feeding. The disappearance kinetics of quizalofop-P-tefuryl in *C. sativa* leaves was determined. Differences in FTIR spectra of *C. sativa* treated to herbicide and aphid feeding were found.

Keywords: herbicide; hemp aphid; fatty acids

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