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**ORAL PRESENTATION THEME B**

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# Molecular and phenotypic diversity in *Alternaria* isolates in North-west regions of India and response of mutant doubled haploid *B. juncea* genotypes to *Alternaria* under artificial inoculation

**Background:** The genetic base for fungal diseases are limited; none of the cultivars of *B. juncea* (Bj) presently being grown in India are resistant/immune to *Alternaria* blight (AB).

**Objective:** To evaluate the diversity spectrum/genetic evolution in AB isolates for understanding host- pathogen interaction and to recover novel genetic source for fungal disease tolerance through haploid mutagenesis.

**Methods:** Single spore isolates of AB (32 *A.brassicae*, 20 *A.brassicicola*, 3 *A.alternata*) from 8 states of North West India, were evaluated for morphological, pathological, cultural, biochemical and molecular characteristics. The doubled haploid (DH) mutant progenies were derived from microspore mutagenesis through ENU/EMS in Bj var. Varuna/Pusa Bold. DI was evaluated (three leaves/plant) on a 0 to 5 scale (0– no symptoms, 0.5- 5%, 1.0- 10%, 1.5- 15%, 2.0 -20%, 3.0- 30%, 4.0- 40%, 5.0- 50%).

**Results:** RAPD analysis revealed a high level of genetic diversity for *A.brassicae* (57-78%), *A.brassicicola* (78-92%), *A.alternata* (89-100%) isolates. *A. brassicae* isolates clustered into four major clades (genetic diversity 0.45-0.65), *A.brassicicola* formed three clades (genetic diversity 0.51-0.76), and *A.alternata* isolates fell in the same clade (GS 0-0.20). Extensive variations were observed in isolates for the parameters studied, however, no correlation could be established among them. The DI for DH mutant progenies was 0.03-1.0 under epiphytotic field conditions and 1.3-3.3 by detached leaf method. Among 40 DH-M4 progenies tested in field (GBPUAT, Pantnagar) under artificial inoculation, four genotypes were moderately resistant (18-30% disease) at 75 DAS. Six genotypes showed 10-12% diseases at pod formation stage. Five lines had no stem infection two weeks before maturity.

**Conclusions:** The study indicated a non- specific relation between the isolates interacting and infecting with different species/varieties of *Brassica* spread over north/north-west regions of India, suggesting that the genes for resistance/tolerance for fungal pathogens may be present in mono-genome *Brassica* and that targeted mutagenesis may be useful for recovering such mutations. The selected DH lines form a valuable gene pool for developing Indian mustard with high tolerance to *Alternaria* blight.

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**080**
**ORAL PRESENTATION THEME B**

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# Canola protein nanoparticles: A promising delivery system for encapsulation of bioactive compounds

**Background:** Encapsulation is an emerging technique to improve solubility, permeability and bioavailability of bioactive compounds after food processing and gastrointestinal digestion. Food proteins are generally recognized as safe (GRAS) biopolymers and have been attracted great attention for using as delivery materials due to versatile functionalities.

**Objectives:** We hypothesised that cruciferin, a major canola protein, due to good functional properties, high denaturation temperature and resistance to gastric enzymes, could be an appropriate carrier for delivery of bioactive compounds. Preparation and evaluation of encapsulating property of cruciferin nanoparticles were our objectives.

**Methods:** Cold gelation and combination with chitosan were used to prepare calcium-induced cruciferin particles (Cru/Ca) and cruciferin/chitosan particles (Cru/Cs). In addition to the size, zeta-potential, morphology, cell toxicity and cell uptake of the particles, their encapsulation properties were also studied using two water-soluble and -insoluble model compounds (brilliant blue and beta-carotene). The protection effects of the encapsulation systems were evaluated in the simulated gastrointestinal tract and also in a heat treatment.

**Results:** The prepared Cru/Ca and Cru/Cs particles were spherical in shape with an average size of ~ 207 and ~ 165 nm, respectively, Negatively-charged Cru/Ca particles (-33 mV) and positively-charged Cru/Cs particles (+20 mV) were stable for more than three weeks at 4°C. The prepared nanoparticles did not show any toxicity to Caco-2 cells after 24 hours of incubation at a concentration of 2.5 mg/mL. Confocal microscope images revealed that fluorescently-labelled particles were uptaken by Caco-2 cells after 6 h incubation. The model compounds were loaded in the particles at encapsulation efficiencies of 80-86%. In vitro release studies showed that both particles were resistant to simulated gastric fluid; while Cru/Ca particles released 70-90% of the model compounds in simulated intestinal fluid, Cru/Cs particles were resistant to intestinal conditions (released only 10-15 % of the compounds). The encapsulation also significantly increased the stability of loaded beta-carotene in a heat treatment (75°C and 30 min) compared to not-encapsulated form.

**Conclusions:** Cruciferin has the ability to form nanoparticles for encapsulation of bioactive food compounds. For the first time, two types of nanoparticles were successfully developed from cruciferin; these particles were appropriate carriers for encapsulation of both hydrophilic and hydrophobic compounds. The cell uptake of the particles revealed that the carriers might also improve the absorption of less-soluble and/or less-permeable compounds in the intestine. This study demonstrated the potential use of cruciferrin as a natural polymer for delivery of bioactive compounds in the gastrointestinal tract to target different sites of action.

**081**
**ORAL PRESENTATION THEME B**

# Parasitoids of swede midge in Saskatchewan

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**Background:** The swede midge was recently accidentally introduced to North America, having been identified in 2000 in Ontario. The midge has had a major impact on canola in eastern Canada, leading even to temporary moratoria on canola production in some areas. Intensive surveys for natural enemies in eastern Canada revealed that swede midge seems free of parasitoid-induced mortality there. More recently swede midge has been found on the prairies, and there swede midge was observed to be attacked.

**Objectives:** To survey parts of Saskatchewan where swede midge occurs to determine which parasitoids are involved and what levels of mortality are caused by these species.

**Methods:** Fused canola flowers with midge larvae inside were collected at five locations in late July, and at nine locations in early September 2014. Flowers were placed on a moist mixture of sand and peat, and midges and parasitoids that emerged were counted. The soil was then kept for three months in cold for diapause, after which emerging insects were again counted.

**Results:** At least two species have been recovered so far, with combined levels of parasitism reaching over 20%. One species, an undescribed *Gastrancistrus* species in the family Pteromalidae, seems to attack swede midge larvae. Another, tentatively identified as *Inostemma* sp. *(Platygasteridae)*, likely attacks eggs.

**Conclusions:** Unlike in eastern Canada (Corlay et al. 2007), on the prairies swede midge is attacked by parasitoids. It may be possible to introduce these species to eastern Canada as biological control agents. However, more information is required about the wasps first, particularly with respect to host specificity.

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**ORAL PRESENTATION THEME B**

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# *AvrLm7* and *AvrLm3* frequency evolution in French *Leptosphaeria maculans* populations

**Background:** Phoma stem canker, caused by the fungus *Leptosphaeria maculans*, is mainly managed through the deployment of resistant varieties. Specific resistance genes (Rlm) are present in commercial varieties and the effectiveness of a given Rlm is a function of the frequency of the corresponding avirulence allele in field populations of the pathogen. Rlm genes exert a strong selection pressure on fungal populations, leading to the selection of virulent isolates and the breakdown of the resistance. However the durability of a given Rlm gene may vary, depending on the plant genetic background (1), the fitness cost linked to the loss of the avirulence gene (2) and agronomic practices (3).

**Objectives:** After the very rapid breakdown of Rlm1 in the 90's in France, it was questioned whether all released Rlm genes could be overcame at the same speed. The Rlm7 resistance gene was introduced in commercial hybrids in France at a time when most (>99.5%) of the isolates possessed the avirulent allele *AvrLm7* (4). This was an appropriate field situation to address this question.

**Methods:** The frequency of virulent isolates was monitored in populations of *L. maculans* at a national scale in 2000-2001, 2010, and 2013. From 8 to 20 sites were sown with varieties devoid of Rlm3 and Rlm7. Isolates were collected form individual leaf lesions from independent plants and phenotyped for their virulence profiles toward Rlm3 and Rlm7 using standard inoculation tests (4).

**Results:** A total of 1787, 577 and 1173 isolates were collected and phenotyped in 2000-2001, 2010 and 2013, respectively. While only one virulent isolates toward Rlm7 (avrLm7) was found in 2000-2001, their mean frequency reached 3.99% in 2010 and 19.8% in 2013. In 2013, the frequency of avrLm7 isolates varied from 0% (Britany) to 45% (Center region). Noticeably, all avrLm7 isolates (all sampling years) but 6 (2013 sampling) were avirulent toward Rlm3. Therefore, only 0.5% of the current French *L. maculans* populations can infect both Rlm3 and Rlm7 varieties.

**Conclusions:** Compared to the Rlm1 breakdown that happened in only 3 growing seasons in France, it took avrLm7 10 years to reach a mean frequency of virulent isolates of 20% at the national level. Large regional variations are however observed in relation to the intensity of oilseed rape cropping and Rlm7 use. This population survey also clearly confirms the negative interaction between the avirulence genes *AvrLm3* and *AvrLm7*, which offers great perspectives for durable management of specific resistance genes in oilseed rape.

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**083**
**ORAL PRESENTATION THEME B**

# Resistance to blackleg disease: From pathogen perception to induction of defense

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**Background:** Blackleg caused by the ascomycete fungus *Leptosphaeria maculans* is a common disease in most canola/ rapeseed *(Brassica napus)* producing countries. After germination of *L. maculans* spores on cotyledons and leaves of young seedlings, hyphae enter and grow between the mesophyll cells. In the absence of race specific resistance (R) genes, the pathogen continues to grow causing tissue collapse and the development of lesions on cotyledons, leaves and at the base of the stem. To date 16 R genes against *L. maculans* from *Brassica* species have been identified but the nature of these genes has remained unknown until recently. Here we present cloning LepR3 and Rlm2 , the first R genes against blackleg to be reported from *Brassica*. The components of the LepR3 and Rlm2 recognition complexes and their down stream signals will be discussed.

**Methodology:** Map-based cloning of LepR3 and Rlm2 was carried out with final complementation of the resistance phenotype produced by transgenic analysis. The *L. maculans* effector AvrLm2 was cloned by genome sequence comparison of 40 *L. maculans* isolates that differentiated for their virulence against Rlm2 plants. The genome of *L. maculans* reference isolate 00-100 was sequenced using Roche 454 and other *L. maculans* isolates were re-sequenced using Illumina technology.

**Results:** We applied map-based cloning approach to isolate LepR3 and Rlm2 resistance genes against blackleg, located within the same genomic interval on chromosome A10 of *B. napus*. LepR3 and Rlm2 are alleles of the same gene encoding for receptor-like proteins (RLP) (1, 2). In addition we discovered that the previously cloned *L. maculans* effector AvrLm1 is recognised by LepR3, revealing an example of a single Avr gene being recognised by two independent R genes (LepR3 and Rlm1). We also cloned *L. maculans* effector AvrLm2 as the pathogen Avr gene that triggers Rlm2-initiated defense response (3). Our investigation into the LepR3 and Rlm2 recognition complexes and downstream signalling pathways revealed that LepR3 and Rlm2 proteins interact with the *Arabidopsis thaliana* and *B. napus* receptor like protein kinase (RLK) SOBIR1. The results of our efforts to identify AvrLm1 target in *B. napus* and to define the gene expression profile of pathogen and host plant genes during infection will be presented.

**Conclusion:** Cloning of LepR3 and Rlm2 resistance genes against blackleg disease and identifying their corresponding Avr genes provide the first insight into the perception of *L. maculans* by its host plant and establish a model system to investigate the components of R gene complex for recognition of *L. maculans* effector proteins, their host targets and downstream signalling pathways.

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**084**
**ORAL PRESENTATION THEME B**

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# Control of pyrethroid resistant pollen beetles – effect of thiacloprid and two pyrethroids on population dynamics

**Background:** Over a long time synthetic pyrethroids were used to control pollen beetles *(Meligethes aeneus F.)* in Germany. The extensive and indiscriminate use of this insecticide class resulted in a high selection pressure on the beetles, ensuing in the formation of resistance, which has spread over many European countries.

**Objectives:** Insecticide applications should reduce yield loss of bud feeding but in addition also the reproduction of the beetles to minimize the infestation pressure in following cultures, e.g. vegetable crops and oilseed rape in the following year. The aim of the study is to provide data for a control strategy for a sustainable reduction of the population density without influencing natural mortality caused by parasitization.

**Methods:** To test the effect of insecticides on the reproduction of pollen beetles field trials were carried out near Braunschweig in 2013 and 2014. The neonicotinoid Biscaya (a.i. thiacloprid) and the pyrethroid insecticides Karate Zeon (lambda-cyhalothrin) and Mavrik (tau-fluvalinat) were sprayed in replicated plots of 0.1 ha size at different BBCH growth stages of winter oilseed rape. In all plots the infestation of overwintered pollen beetles was observed. Additionally the number of eggs per bud and the number of larvae dropping to the ground for pupation was recorded and the new pollen beetle generation emerging from treated and untreated plots was trapped in photoeclectors. The larvae were investigated for parasitization with the key larval parasitoids *Tersilochus heterocerus* and *Phradis* spp.

**Results:** In 2013 Biscaya reduced the number of overwintered pollen beetles until 3 DAA. Egg laying was significantly reduced by application of Biscaya. In addition there was a direct-lethal effect on L1-larvae when Biscaya was applied at BBCH 60/65. As a consequence of both (L1 mortality and egg laying) a reduced number of larvae and new generation pollen beetles hatched. In 2014 these effects of Biscaya on the population dynamic were confirmed. Application of the pyrethroid insecticide Karate Zeon had no effect on overwintered pollen beetles but resulted in an increase of the next generation beetles compared to control values. Mavrik reduced overwintered pollen beetle numbers more than Biscaya and had also an effect on population dynamic but to a lesser extent than Biscaya.

**Conclusions:** By a careful selection and termination of insecticides it seems possible in addition of controlling pest damage also to reduce pest pressure in the following season by influencing population dynamics of pollen beetle.

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**ORAL PRESENTATION THEME B**

# Mapping of quantitative trait loci in Brassica napus lines from Pakistan and China providing resistance to *Sclerotinia sclerotiorum*

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**Background:** Canola is one of several hundred crop species susceptible to the fungal pathogen *Sclerotinia sclerotiorum*. Partial resistance has been identified in a few plant species including some *Brassicas*. After screening of *B. napus* germplasm with isolates representative of the Canadian pathogen population, lines with quantitative resistance were identified originating from Pakistan, China, South Korea and Japan. These sources of resistance together with a reliable disease phenotyping method and high throughput genotyping has facilitated mapping of quantitative trait loci (QTL) conferring resistance against sclerotinia stem rot.

**Objective:** To map major QTLs conferring resistance to *Sclerotinia sclerotiorum* in *B. napus* germplasm and develop molecular markers to aid transfer of this resistance to canola.

**Methods:** Seven doubled haploid (DH) mapping populations segregating for sclerotinia resistance were developed from crosses with a susceptible line and each of three resistant lines, Zhongyou 821 (ZY821, China), PAK54 and PAK93 (Pakistan). All DH lines were genotyped with 6000 SNP and ~700 SSR markers. Linkage maps were constructed for each DH population and an integrated map was generated (Joinmap). DH plants at full flower were phenotyped for sclerotinia resistance by inoculating the main stem with mycelium of a single virulent S. sclerotiorum isolate, #321. Five disease measurements were used for QTL analysis (QTL Cartographer). Meta-analysis was used to align sclerotinia QTLs identified in each DH population as well as QTLs from the literature (BioMercator).

**Results and discussion:** A distinction was made between major and minor QTLs based on phenotypic variation explained (R2), LOD value and number of times each QTL was identified across mapping populations. QTLs were labelled with chromosome number (A1 to C9) and ancestral genomic block (A to X) (Schranz et al. 2006). Major QTLs in PAK54 mapped to chromosome A3F, A6A, A9A, A10R and C1F, which were different from those in PAK93 on chromosome A5J, A7IX, C2X and C6E. A total of eleven QTL were mapped in five DH populations derived from ZY821. Six of these QTLs were present in either PAK54 or PAK93. Most sclerotinia resistance loci mapped to blocks U, F and R. We are currently examining possible duplication of resistance loci resulting from allopolyploid in the *B. napus* genome. Meta-analysis showed that some of the QTLs reported in the literature mapped to our QTLs despite differences in disease phenotyping methods. Seeds of sclerotinia resistant lines from Pakistan, Japan and South Korea are available for cultivar development from Plant Gene Resources of Canada via the corresponding author under a material transfer agreement. Lines from Pakistan have high genetic diversity and would provide diversification in Canadian and Australian canola breeding programs in particular (Gyawali et al. 2013).

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**086**
**ORAL PRESENTATION THEME B**

# Impact of the neonicotinoid insecticide thiamethoxam on the lifespan of the honeybee *Apis mellifera L.* in a large scale study conducted under natural conditions


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**Background:** Thiamethoxam, active ingredient of Cruiser OSR®, is a neonicotinoid systemic insecticide used as seed dressing for oilseed rape protection against early season pests (Elbert et al., 2008). Its use on oilseed rape has been banned in France since 2012 after the release of a semi-field study showing that ingestion of a subletal dose of thiamethoxam resulted in significant homing failure among honeybee foragers (Henry et al., 2012). To determine if those results could be generalized to natural conditions, we carried out a large scale experiment with free foraging honeybees.

**Objectives:** A two-year large-scale study was designed to measure via RFID technology whether honeybee survival could be impacted by exposure to thiamethoxam while foraging freely in a zone partially sowed with oilseed rape seed-dressed with Cruiser OSR®.

**Methods:** In 2013 and 2014, 10 hives were placed in a 150 square kilometers intensive cereal farming system area located near Niort in the West of France (the "Zone Atelier Plaine et Val de Sèvres" research facility). Then, in this area where regular farming practices remained unchanged, 153 ha and 135 ha of seed dressed oilseed rape were sown in 2013 and 2014 respectively. Each hive was settled at a certain distance of the treated oilseed rape fields in order to generate variation in thiamethoxam exposition pressure in the honeybees foraging area. In each hive, 5000 individuals were tagged with RFID microchips in order to monitor honeybees entering and leaving the hives. All oilseed rape fields, whether treated or untreated with thiamethoxam, were georeferenced in order to calculate a thiamethoxam exposure index. Data concerning population levels of adults and larval stages, food storage and diseases were collected in RFID hives to assess the general fitness of the colonies.

**Results:** The thiamethoxam exposure index was calculated on the basis of treated fields' surface and distance from RFID hives. Statistical analyses were performed to assess the influence of this index on tagged honeybees' lifespan. General healthcare of the colonies, population levels, food storage were studied according to thiamethoxam exposure intensity. Influence of the year of experiment and climatic conditions on the percentage of undetected honeybees was also assessed.

**Conclusions:** Our two-year study generated a huge amount of individual data. The performed analyses allowed us to investigate whether honeybee exposure to thiamethoxam via free foraging on oilseed rape crops protected with Cruiser OSR® has a significant impact on individual lifespan.


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**087**
**ORAL PRESENTATION THEME B**

# Screening for resistance to black leg in winter canola germplasm adapted to the U.S. Southern Great Plains

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**Background:** Black leg *(Leptosphaeria maculans)* is a widespread disease of winter canola in the southern Great Plains. Little is known about the reaction of locally adapted germplasm to black leg. Multi-genic resistance to canker development in the field is partially effective depending on the environment. Major-gene resistance, expressed as a hypersensitive response to leaf spot on cotyledons, is controlled by one or more resistance genes (Rlm) that interact in a gene-for-gene manner with avirulence genes (Avr) in the pathogen.

**Objectives:** Entries were screened for field resistance to black leg. Avr alleles in isolates were assessed in order to identify prevalent races of the pathogen. The seedling reaction of germplasm to predominant races was determined to identify major-gene resistance.

**Methods:** Entries were screened from 2011-2013 by inoculating field plots with stubble collected from problem fields and evaluating canker severity after swathing. Avr alleles in local isolates (n=94) were identified by inoculating differentials with one or two Rlm genes or by PCR testing for Avr1, Avr4-7, and Avr6. Differentials included Westar (no Rlm), Quinta (Rlm1), Glacier (Rlm2,Rlm3), and Jet Neuf (Rlm4).

**Results:** In the field, disease incidence was high (50-100%), but canker severity (% stem girdling) averaged less than 50%. Most of the popular glyphosate-tolerant (Roundup Ready; RR) cultivars were more susceptible than conventional (non-glyphosate tolerant) cultivars and hybrids. In the local pathogen population, Avr1 was present in 37% of isolates, Avr2-3 was present in only 10% of isolates, while Avr4-7 and Avr6 were present in 100% of isolates. Based on limited screening of Jet Neuf (Rlm4), most isolates lack Avr4, and Avr7 is likely responsible for the positive PCRs for Avr4-7. A few isolates (n=3) had all Avr alleles (Avr1,2,3,4-7,6) while a few (n=6) only lacked Avr1 (Avr 2,3,4-7,6). Most isolates had fewer Avr alleles and differed only in Avr1. Many isolates (n=32) were Avr1,4-7,6 and most (n=53) were Avr4-7,6. Except for DKW46-15 which was heterogeneous (nearly resistant), RR cultivars and hybrids were susceptible to the predominant races. Some conventional cultivars were resistant or heterogeneous (Kiowa, Sumner, and Wichita), and MH06E10 was resistant to all races. The conventional hybrids Rossini, Visby, Safron, Dimension, and DKSensei were also resistant to all races. The breeding lines KS4426, KS4428, and KS4564 were resistant or heterogeneous to all races. Most (62%) of the 55 entries were susceptible to all races.

**Conclusions:** Most local isolates of *L. maculans* can overcome Rlm1, Rlm2, Rlm3, and probably Rlm4. Rlm6 and Rlm7 should be highly effective. Most popular RR cultivars lack effective Rlm genes and also were the most susceptible in field trials. Several conventional cultivars and breeding lines had non-specific (heterogeneous) resistance to one or more races. Rlm7 is likely present in the conventional entries (n=6) with resistance to all races. There is a need to improve black leg resistance in locally adapted winter canola because most entries (62%) were susceptible to predominant races.

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ORAL PRESENTATION THEME B

# Analysis of quantitative adult resistance to blackleg in canola

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**Background:** Blackleg caused by *Leptospharia maculans* is a highly evolving fungal pathogen affecting the canola industry in Canada, Australia and Europe. Quantitative resistance (horizontal or non-race specific) that shows intermediate resistance can be less effective and difficult to identify compared to qualitative resistance by single dominant genes.

**Objective:** The objective of this study was to evaluate the effectiveness of a previously mapped intermediate blackleg resistance locus at the adult stage under greenhouse and field conditions.

**Methods:** Populations segregating for an intermediate resistance locus were planted and inoculated using cotyledon inoculation. All plants were genotyped using closely linked molecular markers. Blackleg disease symptoms were scored when the plants were fully mature using a 0 (no disease) – 5 (completely susceptible resulting in plant death) scale. Data for the adult stage included the percentage of plants that died before maturity and the disease scores at maturity for surviving plants. Scores for resistant plants ranged from 1 to 3 and susceptible scores ranged from 4 to 5. Near iso-genic lines (NILs) containing the intermediate blackleg resistance locus along with the parents and other controls were planted in a blackleg nursery at the Ian N. Morrison Research Farm Station in Carman, Manitoba. Disease scoring was done at maturity using a 0-5 scale.

**Results:** In the greenhouse, 182 plants were inoculated using a weak isolate. Of these plants, 101 did not harbour the intermediate resistance locus and 94% of the plants died before reaching maturity. Only 2% of these plants survived and received scores ranging from 2-3 (resistant) and 4% of the plants survived and received a score of 4 (severe infection). Of the 81 plants evaluated with the intermediate resistance locus, only 28% died at the early stages, 56% survived with scores 1-3 and 16% with a score of 4. In plants treated with a highly virulent isolate, 100% of the plants without the resistance locus died early in development while 47% of the plants with the resistance locus survived and were rated 1-3. Of the plants treated with another virulent isolate, 64% of plants without the resistance locus died during early development and 27% survived with ratings of 1-3 and 8% survived with a score of 4. Regarding plants with the resistance locus, 24% died during early development and 76% survived with ratings of 1-3. In the field, disease scores ranged from 0.8 to 3.3 (resistant to intermediate) for NILs with the intermediate resistance locus. However, the susceptible control, Westar had an average rating of 4.5 (most plants died before maturity). This demonstrates that the intermediate resistance locus performed well under severe blackleg disease pressure in the field. Because a mixture of isolates was used in the field evaluation, this suggests that the resistance locus confers horizontal resistance.

**Conclusion:** The results indicate that the intermediate resistance locus can confer resistance to multiple isolates in the greenhouse and field, suggesting that this resistance locus has excellent potential in blackleg management in western Canadian canola production regions.

**089**
**ORAL PRESENTATION THEME B**

# *Plasmodiophora brassicae* its genome and associated microbiome

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**Background:** Clubroot caused by *Plasmodiophora brassicae*, is a widespread soil-borne disease with high economical impact on *Brassica* oil- and vegetable crops. *P. brassicae* is phylogenetically located among the Phytomyxids within the poorly known supergroup *Rhizaria. Phytomyxids* consist of two groups: the *Plasmodiophorids* which are parasites of plants and oomycetes, and the *Phagomyxids*, comprising pathogens of sea grass, diatoms, and brown algae.

**Objectives:** In order to enhance our biological understanding of this important plant pathogen we have sequenced its nuclear genome, the mitochondria and monitored the microbial communities associated to this disease in soil, rhizosphere and inside roots of oilseed rape.

**Methods:** Next generation sequencing technologies, along with bioinformatic softwares have been used to establish genome data enabling various comparative genomic analysis, and to elucidate microbial communities associated with healthy and diseased conditions.

**Results:** We will present the 25.5 Mb genome, its developmental stage-specific transcriptomes and the transcriptome of *Spongospora subterranea*, the closely related potato scab pathogen. The *P. brassicae* genome is reduced in metabolic pathways and harbors phytohormones contributing to its gall phenotype. Phylogenetic analysis points to its important role in deciphering evolutionary relationships and gene diversification of early eukaryotes, further demonstrated by its complex mitochondrial genome. A genome that is approximately three times larger than the mitochondria in *S. subterranea* (Gutiérrez et al. 2014). Different microbial communities dominate inside the roots of oilseed rape, its rhizosphere and in the soil of infested or healthy nature. For example, the two bacterial families *Pseudomonadaceae* and *Enterobacteriaceae* are expanded in the diseased clubroots. Details on the structure of the microbiota associated with the clubroot disease will be presented together with other data.

**Conclusions:** The eukaryotic *Rhizaria*, comprises several groups of uncultivable free-living protists such as *Radiolarians, Foraminiferans* and *Gromiids,* as well as the parasitic *Plasmodiophorids* and *Haplosporids*. Here, we provide genome and transcriptome data on two plant pathogenic *Plasmodiophorids*. Together with the microbial communities associated with the clubroot disease we now hold new knowledge useful in the design of new controlling measures against this widespread plant disease.

**References:**

Gutiérrez, P., Bulman, S., Alzate, J, Ortiz, M.C., Marin M. 2014. Mitochondrial genome sequence of the potato powdery scab pathogen *Spongospora subterranea*. Mitoch. DNA. doi 10.3109.

090
ORAL PRESENTATION THEME B

# Genetic resistance to blackleg in the Canadian banola *(B. napus)* germplasm and the breakdown of the dominant *Rlm3* by the *L. maculans* pathogen population

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**Background:** *Leptosphaeria maculans* is the causal agent of blackleg (aka phoma stem canker), an economically important disease of *Brassica napus* (canola/oilseed rape). This pathogen has led to epidemics in France and Australia and is now a growing concern in the Canadian Canola *(Brassica napus)* industry where genetic resistance in major varieties has long provided an effective means of disease control. Both seedling resistance controlled by major resistance genes (*Rlm* genes) and adult plant resistance mediated by quantitative resistance (minor) genes to *L. maculans* have been identified in canola varieties. Tighter rotations and increased acreage driven by the economic returns of canola have led to the emergence of new virulent races. Major gene resistance to blackleg is analogous to the gene for gene interaction model whereby *Rlm* genes defend against isolates with corresponding *Avrlm* genes. However, host genetic resistance can be overcome with population shifts and the emergence of new races of the pathogen.

**Objectives:** The aim of this study was to identify the major seedling resistance genes carried in the Canadian canola *(B. napus)* germplasm as well as well the predominant avirulence genes carried by the Canadian Blackleg pathogen population.

**Methods:** To identify host resistance to blackleg, a set of genetically characterized *L. maculans* isolates were employed to identify major resistance genes (*Rlm* genes) in 206 Canadian canola varieties/lines. 104 of these canola varieties/lines were further evaluated for adult plant resistance (APR) under controlled conditions. To identify pathogen race structure, 674 *L. maculans* isolates isolated from stubble collected in 2010 and 2011 across Western Canada were characterized at ten avirulence genes using a set of *Brassica* lines carrying known Rlm genes.

**Results:** On the host side, the results indicate that 85% of tested canola varieties/lines carry seedling resistance. However, except for *Rlm3* which was present in the majority of lines tested, the rest of *Rlm* genes were rarely detected. Adult plant resistance to blackleg was identified in 56% of tested varieties/lines. On the pathogen side, a total of 55 races were detected with two dominant races accounting for 45% of the pathogen population. *Avrlm6* and *Avrlm7* were the dominant avirulence genes and were present in >85% of isolates and *Avrlm3*, *Avrlm9*, and *AvrLepR2* were the least observable and present in <10% of isolates. The above results indicate the breakdown of *Rlm3* resistance in Western Canada most probably due to selection pressure in response to the presence of this single resistance gene in most varieties.

**Conclusions:** Genetic resistance to blackleg in the Canadian Canola germplasm is derived from a combination of adult plant resistance and a few seedling resistance genes with heavy reliance on *Rlm3*. Most of the blackleg pathogen population is now virulent on *Rlm3* and while there are many races, a few races account for the majority of the pathogen population. The gradual increase in blackleg disease incidence and severity in western Canadian disease surveys indicates the need to incorporate both adult plant resistance and diverse seedling resistance into commercial varieties. Management strategies such as pathogen monitoring and cultivar rotation are key factors in preventing significant yield loss over the long term.

**091**
**ORAL PRESENTATION THEME B**

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# Molecular mapping of clubroot resistance in spring *Brassica napus* canola with resistance introgressed from the European winter canola cv. 'Mendel'

**Background:** Clubroot disease caused by the soil-borne pathogen *Plasmiodiophora brassicae* Woronin is a major threat to the production of *Brassica* crops worldwide. Breeding for resistance requires the identification of resistance sources and successful introgression of the resistance gene(s) into crop germplasm (for review, see Rahman et al. 2014). The European winter canola cv. 'Mendel' show resistance to many *P. brassicae* isolates including pathotypes 3 which is prevalent in Canada (Rahman et al. 2011).

**Objectives:** The identification and the genomic localization of molecular markers linked to clubroot disease resistance gene(s) in spring canola lines derived from the European winter *B. napus* cv. 'Mendel' will help to understand the mechanism of the resistance and also aid in the marker assisted breeding of this crop.

**Methods:** To introgress clubroot resistance into Canadian spring *B. napus* canola, crosses between Canadian spring canola and European winter canola cv. 'Mendel' were made and several resistant lines were developed through pedigree breeding. Two of the resistant lines were further crossed to the clubroot susceptible spring canola line A07-26NR and doubled haploid (DH) populations were produced from the F1's. Simple sequence repeat (SSR) markers were used to identify markers linked to the resistance in two DH populations as well as in segregating F3 and F4 families derived from the original Canadian spring canola × 'Mendel' crosses.

**Results:** The inheritance and molecular mapping studies suggested that at least one dominant gene is involved in the control of clubroot resistance derived from the European winter *B. napus* cv. 'Mendel'. The resistance gene was mapped to the A3 chromosome of *Brassica rapa*.

**Conclusions:** We identified two SSR markers linked to the clubroot resistance gene. Markers identified in this study can be used in marker assisted breeding as well as pyramiding of multiple clubroot resistance genes.

**References:**

Rahman H, Shakir A, Hassan MJ (2011) Breeding for clubroot resistant spring canola *(Brassica napus L.)* for the Canadian prairies: Can the European winter canola cv. 'Mendel' be used as a source of resistance? Can J Plant Sci 91: 447-458 doi:10.4141/CJPS10073

Rahman H, Peng G, Yu F, Falk KC, Kulkarni M, Selvaraj G (2014) Genetics and breeding for clubroot resistance in Canadian spring canola *(Brassica napus L)*. Can J of Plant Path 36: (1): 122-134. DOI:10.1080/07060661.2013.862571

**092**
**ORAL PRESENTATION THEME B**

# Comparative genomics facilitate cloning of *Leptosphaeria maculans* avirulence gene *AvrLm2*

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**Background:** Blackleg caused by *Leptosphaeria maculans* is a major disease of the oilseed crop *Brassica napus* (canola/oilseed rape) and other *Brassica* crops worldwide. Race identification of the pathogen through cloning and marker development of avirulence genes is a crucial step for the disease management. Map-based cloning has been successfully applied to clone five *L. maculans avirulence* genes. However this approach is time consuming and has limitations such as incompatibility of desired parental isolates for crossing. With the advent of next generation sequencing and the availability of whole genome sequences of *L. maculans*, a combination of genetic mapping, high-throughput phenotyping and intraspecies comparative genomics can facilitate the identification of avirulence genes.

**Objectives:** Cloning *L. maculans AvrLm2* gene corresponding to the race specific *B. napus* resistance gene *Rlm2*.

**Methods:** *AvrLm2* was previously reported to be located within the *AvrLm1*-AvrLm6 gene cluster of *L. maculnas* genome (Fudal et al., 2007).To compare *AvrLm2* genomic interval, we re-sequenced *L. maculnas* isolates using Illumina sequencing platform. Sequence reads for each isolates were mapped to the reference genome v23.1.3 using Bowtie2 and visualized using GBrowse 2.0. We then searched the *AvrLm1-AvrLm6* genome interval for SNP(s) (single nucleotide polymorphism).

**Results:** Three SNPs coincident with the *AvrLm2* phenotype were identified in the predicted effector gene *LmCys1*. Complementation of a virulent isolate with *LmCys1*, as the candidate *AvrLm2* allele, restored the avirulent phenotype on *Rlm2*-containing *B. napus* lines proving that the predicted effector gene is *AvrLm2*. Mutation analysis showed that only the non-synonymous changes of G397->A/C397 or G398->A398, both of which lead to a change of amino acid at Gly133, were responsible for the loss of *Rlm2*-mediated recognition specificity. Expression pattern of *AvrLm2* alleles (Avr and avr) were similar during infection and picked at 5 days after inoculation.

**Conclusions:** The cloning of *AvrLm2* described here provides an example for the rapid cloning of effector genes through comparative genomics and as an alternative or complementary approach to map-based cloning. *AvrLm2* encodes a small cysteine-rich protein with low similarity to other proteins in the public databases. Unlike other avirulence genes, *AvrLm2* resides in a small GC-island within an AT-rich isochore of the genome, and was never found completely deleted in virulent isolates.

**References:**

Fudal, I., Ross, S., Gout, L., Blaise, F., Kuhn, M. L., Eckert, M. R., Cattolico, L., Balesdent, M. H. and Rouxel, T. 2007. Heterochromatin-Like regions as ecological niches for avirulence genes in the *Leptosphaeria maculans* genome, map-based cloning of AvrLm6. Mol. Plant-Microbe Interact. 20: 459-470.

**093**
**ORAL PRESENTATION THEME B**

# Spread of clubroot on canola in Canada, 2003 2014

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**Background:** Clubroot caused by *Plasmodiophora brassicae* is an important disease in oilseed rape *(Brassica napus)* crops in Europe. However, it had not been reported on canola-quality *B. napus* on the Canadian prairies until 2003. The initial discovery in 2003 consisted of a small cluster of infested fields (Tewari et al. 2005).

**Objectives:** To summarize the spread of *P. brassicae* across the Canadian prairies, and contrast the rapid movement on the prairies with slow dispersal at a site in southern Ontario.

**Methods:** Surveys for clubroot on canola were initiated in 2004 in the province of Alberta. The initial surveys were relatively small, involving just 41 fields in 2004, 112 fields in 2005, and 250 fields in 2006. Since 2008, more than 400 commercial canola crops in central and southern Alberta were examined each year, and individual counties conducted additional surveys. Annual surveys of more than 100 canola crops in both Saskatchewan and Manitoba were conducted over the past six years. At the Muck Crops Research Station (MCRS) of the University of Guelph, located in the Holland Marsh in Ontario, one block is heavily infested with clubroot (90 100% severity), but susceptible hosts in adjacent blocks develop moderate or even no clubroot. In 2014, replicated trials of clubroot-susceptible brassica vegetables in a trial 50 m from the infested block and at two nearby research sites were rated for clubroot to assess the distribution of *P. brassicae* at sites that are accessed routinely by equipment and workers from the MCRS, with no sanitation measures to restrict transfer of inoculum.

**Results:** *P. brassicae* has spread across large areas of Alberta, with more than 1800 fields infested. Isolated fields with trace levels of clubroot have been identified in Saskatchewan and Manitoba, and severe clubroot has recently been reported in North Dakota (Chittem et al. 2014). In contrast, clubroot severity at the site adjacent to the infested block at the MCRS was < 50%, < 20% 1 km away, and 0% 4 km away, which indicates that spore movement is quite limited.

**Conclusions:** *P. brassicae* is spreading rapidly on the Canadian prairies via both short- and long-distance dispersal mechanisms. Pathogen spread at MCRS is much more limited, and more similar to spread in Germany, where there is little movement of inoculum of *P. brassicae* from region to region (Strehlow et al. 2014). This difference may be related, at least in part, to the size of the inoculum source (field size, field proximity, resting spore concentration; Gossen et al. 2013), patterns of movement of equipment, and for MCRS, possibly soil type.

**References:**

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Tewari, J. P., S. E. Strelkov, D. Orchard, M. Hartman, R. M. Lange, T. K. Turkington. 2005. Plant Dis. 67:758-762.

**094**
**ORAL PRESENTATION THEME B**

# Genetic dissection of resistance to *Verticillium longisporum* in *Brassica* and *Arabidopsis*

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*Verticillium longisporum* is a soil-borne fungus infecting cruciferous hosts. It penetrates host roots, grows towards the xylem and spreads within the xylem vessels to colonise the whole plant. Infected plants show a complex pattern of disease symptoms and developmental reactions, incl. premature seed ripening, chlorosis or stunting. *Brassica* species as well as *Arabidopsis thaliana* are hosts of *Verticillium longisporum*. The aim of our study is to identify genes controlling disease resistance parameters and implications of host development for pathogenesis and defense.

Genetic mapping in *Arabidopsis* and *Brassica alboglabra* revealed a number of QTL explaining the variation for different resistance parameters. In *B. alboglabra*, a closely related species of *B. oleracea*, two major and one minor QTL were found that explained significant proportions of the variation for the degree of fungal colonisation and/ or AUDPC in F3 families. QTL for the control of different stunting parameters showed some overlap with QTL for AUDPC or colonisation. Two major QTL controlling differences in flowering time were identified on C8 and C9 that did not show interdependence to disease resistance. As for *B. alboglabra*, fungal shoot colonisation was the most reliable resistance parameter in repeated experiments with *Arabidopsis*. Resistance to *Verticillium*-induced stunting was inherited more independent from resistance to shoot colonisation than in *B. alboglabra*. QTL for colonisation resistance did show linkage with QTL that controlled development or with the morphological marker erecta. Fine-mapping of different disease parameters in the erecta region in near-isogenic lines revealed a close linkage of loci controlling either stunting or colonisation resistance. A tailor-made (tm) NIL was created that represented the colonization resistance QTL vec1 from the erecta region in a fixed genetic background. µ-array experiments revealed striking differences in gene expression patterns depending on the presence of the QTL and the inoculation. Candidate genes from the QTL region have been identified and cloned. Expression patterns revealed the involvement of different resistance pathways in vec1-controlled defense. Possible synteny between known QTL for *Verticillium* resistance in *Brassica* and the candidate genes from *Arabidopsis* will be presented. Defense related hormones indicated that SA played a role in stunting resistance while JA contents seemed to play a role in colonization resistance.

*Verticillium* resistance is a complex, quantitative trait based on often subtle effects of genes that are related not only to defense responses. Further studies of transgenics expressing the cloned candidate genes will reveal their role in resistance to *V. longisporum* and will support the breeding for resistance in oilseed rape.

**095**
**ORAL PRESENTATION THEME B**

# Clubroot resistance breeding in canola through gene mapping and marker assisted selection

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**Background:** Clubroot caused by an obligate biotrophic parasite, Plasmodiophora brassicae is one of the most economically important diseases of canola/rapeseed and other brassica vegetables in the world. The origin of clubroot disease is not clearly known, it appears as old as its host. Currently, clubroot disease is well spread in more than 60 Brassica crop growing countries. In Canada, clubroot disease is spreading rapidly in canola/rapeseed crop in the Prairie Provinces. Soil-borne and obligate biotroph nature of the pathogen as well as long term viability of resting spores pose big challenges in controlling clubroot disease through various management practices such as chemical control, biological control and other agronomic. Breeding clubroot resistant cultivars is therefore more reliable, cost-effective and environmentally sustainable. In various sources of clubroot resistance in Brassica species, European turnips are the best ones so far, which can be used for breeding clubroot resistant canola/rapeseed cultivars. To use the clubroot resistant sources effectively, it is necessary to map/fine map the resistance genes to develop molecular markers that can be used for marker assisted selection (MAS) in canola/rapeseed.

**Objectives:** Novel resistance genes in European turnips need to be mapped and molecular markers can be developed to transfer these resistance loci in canola/rapeseed cultivars through MAS.

**Methods:** In our clubroot research program, initially we identified the European clubroot differential (ECD) set having high levels of resistance to Canadian field isolates of P. brassicae. We developed segregating populations using all turnip accessions in the ECD set for gene mapping and molecular marker development.

**Results:** In total, three resistance loci have been identified and they represent all major loci in the ECD set. All near-iso genic lines for all resistance loci have been developed and molecular markers closely linked to each clubroot resistance locus have been developed for MAS. These near-iso genic lines are being used to investigate interactions of each locus with different pathotypes. Further, resynthesized B. napus lines developed to integrate these clubroot resistance loci from European turnips to Canadian canola and advance backcross progenies have been developed through MAS. Meanwhile, all clubroot resistance loci were introgressed in canola (B. napus) from progenitor species (B. rapa) and subsequent backcrossing. The results showed clubroot resistance loci were fully functional in canola.

**Conclusion:** In our clubroot research project, we mapped three novel clubroot resistance loci including eight haplotypes and introduced these eight haplotypes of clubroot resistance into canola. Molecular markers have been developed and used for MAS in canola/rapeseed breeding for clubroot resistance gene introgression. In addition, multiple clubroot resistance loci can be pyramid through MAS to develop high levels of clubroot resistance in canola/rapeseed.

# Identification of QTLs for resistance to *Sclerotinia sclerotiorum* and their Interactions with flowering time QTLs in *Brassica napus*

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**Background:** Stem rot caused by *Sclerotinia sclerotiorum* is a major yield-limiting factor in canola production and its incidence in field closely associates with flowering time. There is an urgent need to develop molecular markers linked to resistance for breeding.

**Objectives:** this study is to develop molecular markers linked to *Sclerotinia* resistance.

**Methods:** This study used a *B. napus* RILs population that derived from a cross between 888-5 (a susceptible and early flowering line) × M083 (a resistant and later flowering line) to construct a high dense SNP genetic map through employing the *B. napus* 60K SNP infinium iSelect®HD BeadChip. Resistances to *S. sclertiotum* were identified using three identification methods (Liu et al. 2005), of which, field methods was performed in multiple environments.

**Results:** The genetic map comprised of 9,278 SNPs, and spanned 4071cM of the *B. napus* genome. For phenotyping, resistance evaluation methods included stem inoculation with the fungal mycelial plugs from the edge of colonies cultured in PDA media, artificial field disease nursery identification (5 years) and natural infection tests in 3 locations, and Correlation coefficients among the three methods were significantly positive, and among multi-environments in field natural infection were also positive ($P<0.01$). For flowering time, phenotyping was done in 6 natural infection tests of 3 locations. The flowering time was negatively and significantly related with disease index in all the environments. QTL mapping was performed with two methods: single environment and multi-environment detections. A total of 31 QTLs for resistance were detected on A2, A3, A4, A7, A8, A9, A10, C2, C3, C6, C7 and C8 linkage groups, each explaining 6.14 % to 42.61 % of phenotypic variation. Of them, 12 QTLs were repeatedly found in multiple environments. Major QTL qSSE2-1, explaining 10.50%-27.30% of the phenotypic variation across the 8 environments, and showed independence of environment. Meanwhile, total 21 putative QTLs for flowering time were found on A2, A3, A9, A10, C1, C3 and C7 linkage groups, each explaining 6.13 %-34.50 % phenotypic variation. Among them, 7 QTLs were identified in more than one environment. Three major QTLs (qFT2-3, qFT2-4 and qFTE2-1) were found in multiple environments, contributing 16.40%-34.50% of the phenotypic variation. And the major QTL qSSE2-1 for S. sclerotiorum resistance linked with a major QTL qFTE2-1 of flowering time. In addition, one microsynteny of QTL regions between A2 and C2 was found. According to the direction of QTLs additive effect of this region, the susceptibility to *S. sclertiotum* was linked to early flowering time.

**Conclusions:** The molecular markers for *Sclerotinia* resistance and flowering time and their linkage relationship will be of benefit to improving the efficiency of resistance breeding in oilseed rape.

**Reference:**

Liu S, Wang H, Zhang J, Fitt BDL, Xu Z, Evans N, Liu Y, Yang W, Guo X, 2005. In vitro mutation and selection of doubled-haploid *Brassica napus* lines with improved resistance to *Sclerotinia sclerotiorum*. Plant Cell Reports 24:133-144.

**097**
**ORAL PRESENTATION THEME B**

# *Leptosphaeria maculans* – An aggressive OSR pathogen and air pollutant

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**Background:** Blackleg is a damaging disease of oilseed rape worldwide, caused by *Leptosphaeria maculans* and *L. biglobosa*. The disease is initiated by airborne ascospores, released from fruiting bodies formed on infected stubble from previous seasons. Ascospores are discharged in conducive weather conditions, starting from late summer onwards in Europe or the following spring in North America. Leaf infections initiated by ascospores lead to endophytic colonization of petioles. The formation of severe lesions at stem bases and root collars leads to pre-mature seed ripening, lodging and yield losses. The release of ascospores greatly depends on weather conditions. Disease control is at its most efficient not later than two weeks after mass ascospore release. PCR methods enable the discrimination of *L. maculans* propagules from those of other fungal species, including *L. biglobosa*. Quantitative real-time PCR has facilitated the quantification of the number of spores of both species, which helps to control the disease. Recent advances in genome and proteome analyses are currently enabling us to extend our knowledge of the genes and proteins of *L. maculans*.

**Objectives:** The aim was to compare the ascospore release patterns of *Leptosphaeria* spp. and incidence of phoma stem canker disease of OSR in Poland and the UK, and elaborate forecasting models for ascospore release of (i) all species of *Leptosphaeria*, and (ii) *L. maculans* and *L. biglobosa*. We have also tested the hypothesis that airborne *Leptosphaeria* ascospores can partially explain respiratory problems encountered in the autumn ('autumn asthma').

**Results:** In the last 10 years, the highest concentrations of the ascospores of *L. maculans* and *L. biglobosa* measured at ground level reached ca. 300 s m-3 in Poland and 3350 s m-3 in the UK. To predict the date of the first sudden rise of ascospores of this species complex a comprehensive forecasting model, SimAsco, has been developed. The predictive quality of the model was evaluated using the dataset collected at 12 site-years in Poland (2007-2012). SimAsco proved to be effective, with an efficiency of 0.74. A forecasting model was also elaborated for Poland and the UK to monitor the fluctuations of ascospore concentrations of all species of *Leptosphaeria* in the air. In addition, based on detailed in silico analyses, we have demonstrated that *L. maculans* produces proteins with high identity and similarity to commonly-known aeroallergens of several other well-characterized moulds. There were 81 proteins exceeding 50% amino acid identity, which significantly exceeded the allergen amino acid sequence identity thresholds recommended by FAO/WHO for allergenic proteins in food. High concentrations of *Leptosphaeria* spp. ascospores in the autumn and the postulated allergenicity of their proteins make this fungal genus a possible 'culprit' contributing putatively to respiratory problems.

**Conclusion:** *Leptosphaeria maculans* is not only the aggressive pathogen of OSR, but it is also an air pollutant that produces numerous allergenic proteins

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**ORAL PRESENTATION THEME B**

# *Brassica rapa* genome survey for genes corresponding to *B. juncea* QTL for white rust resistance

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**Background:** *Brassica juncea* (Indian mustard) is a major oilseed *brassica* with white rust one of the important disease in mustard growing areas of India. QTLs for white rust resistance were reported in *B. juncea, B.rapa, B. napus* and *Arabidopsis thaliana*, genes for resistance were identified only from *A. thaliana*. In *B. juncea*, QTLs showed monogenic inheritance and *A. thaliana* gene sequence based Intron Polymorphic markers were flanking the white rust resistance QTLs on `A` genome of *B. juncea* (Punjabi et al, 2010). Availability of *B. rapa* genome sequence (A genome) provided the opportunity to study white rust resistance QTLs of *B. juncea*.

**Objectives:** In silico genome wide survey of *B. rapa* genome sequence for identification of resistance related genes corresponding to *B. juncea* QTL for white rust resistance.

**Methods:** QTL for complete resistance, AcB1-A5.1 was mapped earlier on chromosome A05 of *B. juncea*, spanning a distance of 6.4cM. The corresponding region to this QTL from *A. thaliana* genome was used to search similar regions in the *B. rapa* whole genome sequence, using BLASTN search tool available online at *Brassica* database (BRAD), similar search was performed with Phytozome portal. BLASTN search was performed by dividing the corresponding region of *Arabidopsis* into 15 blocks of 20 loci each. Protein domains were predicted for the candidate resistance related genes using Pfam and genes were grouped according to domain similarity.

**Results:** The QTL AcB1-A5.1 corresponded to a 703 kb region in *A. thaliana* genome, this region matched to homologous sequences on three chromosomes of *B. rapa* i.e A03, A04 and A05. The BLAST results of both *Brassica* database and phytozome were compared; phytozome gave better results with larger regions of homology. A 385kb region on chromosome A03, 634 kb on chromosome A04 and 871kb region on chromosome A05 of *B. rapa* genome sequence were found corresponding to *A. thaliana* region. The arrangement of homologous blocks on chromosome A05 was inverted while in chromosome A03 and A04, the arrangement was the same as in *Arabidopsis*. Total fourteen resistance related genes were identified on these three chromosomes, which were further divided into three classes based on protein domains.

**Conclusion:** Use of native genes into particular species provide less penalty on performance of agronomically high yielding varieties, also it is easy to transfer genes in same or related species using marker assisted breeding. Confirmation of identified genes will be useful in developing high performing *B. juncea* varieties with resistance to white rust.

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**ORAL PRESENTATION THEME B**

# Epidemiology and management of Sclerotinia stem rot in canola

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**Background:** Canola *(B. napus L.)* is the major oilseed crop in Australia. Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* (SSR) is a major disease affecting canola production in most of the canola growing regions in the world (Bradley et al. 2006, Turkington et al. 1993). In Australia, this disease has emerged as a serious problem over the last few years. Losses from SSR in Western Australia have been estimated at A$59 and 23 million in 2013 and 2014 seasons respectively. Various fungicide products are registered for the management of Sclerotinia in Australia, however, information is required under what conditions the fungicide usage is most cost-effective to curtail the disease.

**Objectives:** Three major objectives of the investigations were to determine the efficacy of fungicides, optimize the timing of foliar fungicide application and characterization of environmental conditions involved in the spore production and disease development.

**Methods:** Field trials were conducted in the canola growing regions of Western Australia during 2010-2014 to determine the efficacy of various fungicides and the optimum timing of application of foliar fungicide to manage Sclerotinia stem rot in canola. Fungicides were applied at various bloom stages at the recommended rates. Per cent petal infection was recorded each year. Weather parameters including rainfall, temperature and humidity were recorded from the nearest weather stations. Sclerotinia assessments were made on 50 plants per plot two weeks before harvest, and disease incidence was calculated. Plots were harvested for yield. Data were analysed by ANOVA or REML (Residual maximum likelihood) using Genstat release 16.

**Results:** Various fungicide products were effective in the control of Sclerotinia stem rot, however, yield benefits were achieved when fungicide spray applications were applied synchronizing with the timing of onset of disease epidemics. For example, in a year where spore release was delayed, late fungicide application applied at, or after, 50% bloom significantly reduced the Sclerotinia stem rot incidence and significantly improved the seed yield with highest gross margin compared to nil treatment. Very early spray at 6-7 leaf stage (prior to commencement of flowering) did not significantly increase yield and gave a negative change in gross margin. Trial data indicated that rainfall and relative humidity were the two key drivers in the development of Sclerotinia and threshold values were determined. This is a major breakthrough in determining the risk factors and consequently assisting growers in making fungicide spray decisions.

**Conclusions:** Timing of fungicide application should coincide with the onset of spore release but also taking into account whether subsequent seasonal conditions will be conducive for disease development. The role of weather conditions in disease development will be further discussed.

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# Improved management of light leaf spot by understanding the structure of *Pyrenopeziza brassicae* populations

**Background:** Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicae*, is currently the major disease problem in oilseed rape *(Brassica napus L.)* production in the UK and also affects vegetable *brassicas* such as cabbage, cauliflower and Brussels sprouts. The disease was considered a problem in Scotland and North England but has substantially increased importance in all parts of England over the last decade. Furthermore oilseed rape breeders have seen more light leaf spot in the northern parts of Europe. Due to the polycyclic nature of the disease, the pathogen has the potential to adapt to an environment (McDonald & Linde, 2002). Effective control of light leaf spot to reduce yield and economic losses is difficult to achieve. Fungicide control of the disease in crops is difficult since fungicides must be applied when the pathogen is growing asymptomatically in plant tissues (Figueroa et al. 1994). Additionally, decreased sensitivity to azole fungicides has been reported (Carter et al. 2013). Exploiting plant resistance against the pathogen could help control the disease but current commercial cultivars show poor resistance (HGCA recommended lists, http://www.hgca.com/varieties). The structure of the pathogen population has previously been described as highly genetically diverse (Majer et al. 1998)

**Objective:** To determine the population structure and to study the host range of *Pyrenopeziza brassicae*.

**Methods:** Field assessments with 10 cultivars were done at five locations in England and one location in Scotland to discover possible shifts in pathogenicity towards specific cultivars. Furthermore, oilseed rape and vegetable brassicas were inoculated with *P. brassicae* to identify the host range and possible gene-for-gene interactions.

**Results:** Oilseed rape cultivars showed differences in susceptibility to *P. brassicae* at different locations. Cultivar differences were also recorded in in planta experiments.

**Conclusions:** The results suggest that different pathogen populations may be present at different locations. With increased information about pathogen populations regional advice for deployment of cultivars can be given to farmers for a more effective use of cultivar resistance.

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# Genome wide association analysis and differential expression analysis of resistance to *Sclerotinia* stem rot in *Brassica napus*

**Background:** *Sclerotinia sclerotiorum* is a necrotrophic pathogen, which has no specific host and infects more than 400 plant species. *Brassica napus* is one of the most important oil crops in China, stem rot caused by *Sclerotinia sclerotiorum* is the major disease, leading to yield and quality loss of rapeseed. Resistance to *S. sclerotiorum* showed quantitative inheritance with additive effect and medium heritability, some QTL were found through QTL mapping.

**Objectives:** To understand the genetic mechanism of *S. sclerotiorum* resistance in *B. napus* and identify the candidate genes resistance to *S. sclerotiorum* through GWAS and transcriptome analysis.

**Methods:** Totally 347 *B. napus* were collected in the study and cultivated in southwest university of Beibei, Chongqing, China in 2012 and 2013, the *Sclerotinia* resistance of detached stem inoculation was evaluated according to Mei et al. (2012). SNP genotyping was performed using the *Brassica* 60K Illumina SNP array, and significant associated SNP loci were identified. In addition, transcriptome sequencing of stem in resistant and susceptible *B. napus* after inoculation with *S. sclerotiorum* was conducted.

**Results:** A total of 18 significant associations were identified for stem resistance on A8 and C6. SNPs on A8 were located in a 409 kb haplotype block and association signals detected on C6 were consistent with previous studies of QTL mapping. After *B. napus* infected by *S. sclerotium*, photosynthesis, glyoxalic acid and carbon metabolism were suppressed, while secondary metabolites, sulfur metabolism, especially GSH and glucosinolates were synthesized and immune system was activated, and these systems played an important role in defense response. Numerous ERF and WRKY genes were also found and mostly were up-regulated. Besides, specific genes related with jasmonic acid pathway, lignin biosynthesis, defense response, signal transduction and transcription factors responsible for stem resistance were found.

**Conclusions:** Combining the SNP-trait association and transcriptome sequencing results, 24 common genes were found, including a tau class glutathione S-transferase (GSTU) gene cluster. This study was useful for further gene identification and function analysis.

# The importance of the low temperature threshold for clubroot development on canola

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**Background:** Many studies have been conducted to understand the relationship between temperature, infection by *Plasmodiophora brassicae*, and the development of clubroot. The focus has been on optimum temperatures, but research has also shown that seeding into cool soils can reduce symptoms, and cool temperatures prior to harvest suppress symptoms. A greater understanding of the low temperature threshold that prevents or inhibits clubroot development will improve disease forecasting and timing of seeding of crops and research trials.

**Objective:** To determine the relationship between temperature and clubroot symptom development, with emphasis on identifying the low temperature threshold.

**Methods:** Each year from 2011 2014, a field trial at the Muck Crops Research Station of the University of Guelph, Bradford ON was seeded to canola cv. InVigor 5030 at a site naturally infested with *P. brassicae*. The study was arranged in a randomized complete block design with four replicates. The treatments were seeding at 2-week intervals from early May until late June, to provide a wide range of temperature and soil moisture conditions. Plants were harvested 4 and 6 weeks after seeding and clubroot severity was assessed. Daily rainfall and mean soil and air temperatures were recorded. Correlation analysis and stepwise regression were used to determine the relationship between clubroot severity, and soil and air temperature, rainfall and degree days with a base of 12.5, 14 and 17 °C.

**Results:** Degree day accumulation (base 14 °C) in the first 2 or 3 weeks after seeding was the best indicator of clubroot incidence or severity at 6 weeks after seeding, especially where soil moisture was not limiting or excessive. Degree day calculations with a base threshold of 12.5 and 17 had lower correlation coefficients than day degrees with a 14 °C base. Air temperature was more closely correlated to clubroot development than soil temperature.

**Conclusions:** Degree day accumulation with a minimum threshold of 14 °C was best for predicting clubroot development. However, the number of days when mean temperatures are above this threshold may be a better indicator of clubroot development than either mean temperature or degree days. These results are consistent with controlled environment studies, which showed that primary infection of root hairs occurred at 10 °C, but that cortical infection did not, and that only low levels of cortical infection occurred at 15 °C (Sharma et al. 2011 a,b).

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# Growing winter oilseed rape without neonicotinoid seed treatments – The UK perspective

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**Background:** On 1 December 2013 a restriction adopted by the European Commission on the use of clothianidin, imidacloprid and thiamethoxam (neonicotinoid insecticides) came into force (EFSA, 2013). It addresses the use of pesticides in the treatment of crops attractive to bee pollinators and also for cereals. The restriction was adopted in order to protect, or at least to assess the impact of withdrawal, on bee pollinators. This paper gives an overview of work commissioned by AHDB Cereals and Oilseeds on the implications of the restriction on the neonicotinoids, focusing on crop protection of winter oilseed rape in the UK in the 2014/2015 cropping season.

**Objectives:**
- To confirming whether pyrethroid resistance is present in UK populations of cabbage stem flea beetle (CSFB)
- To assess the incidence and severity of CSFB in winter oilseed rape
- To assess CSFB larval pressures
- To determine the area of oilseed rape lost to CSFB

**Methods:** Twelve samples containing 289 beetles, spanning six counties were received, from growers with suspected resistance, and tested for the presence of the knock down resistance (kdr) mutation.

County level information on the incidence and severity of CSFB during the period 22-29 September 2014 was provided by a network of 23 local agronomists from ADAS. Assessments were based on approximately 32,000ha of winter oilseed rape crops, equivalent to 5% of the national area.

In February 2014, CSFB larval assessments are being carried out by ADAS in counties previously identified as having the highest proportion of oilseed rape crops above the adult CSFB treatment thresholds (HGCA, 2014).

Around 3,500 arable farmers received a winter planting survey in November 2014. It included questions to help assess the impact of the neonicotinoid restrictions on oilseed rape planting and cabbage stem flea beetle damage.

**Results:** CSFB resistance testing confirmed the kdr mutation was present. Enhanced metabolic resistance was also detected.

An estimated 3% (equivalent to 18,000ha), of the national area of winter oilseed rape had been lost by the end of September 2015 due to CSFB. Counties in the East of England were estimated to have been worst affected including Hampshire/Surrey where loses of up to 28% were estimated.

Larvae assessments and planting survey results will not be available until spring 2015.

**Conclusions:** During crop establishment pyrethroid insecticides were the only available option for controlling CSFB. However the confirmation of resistance to pyrethroids is likely to have created more serious issues in controlling this pest.

Crop losses were recorded mainly in the East of England. Here, many crops were drilled in late August/early September. A prolonged period of dry weather resulted in crops remaining at the cotyledon stage for longer. Optimum conditions during early stages of growth may give an untreated crop a better chance to grow away from CSFB attack.

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# Resistance of selected oilseed rape cultivars registered in Poland to stem canker of brassicas *(Leptosphaeria* ssp.*)*

**Background:** Among other fungal pathogens, oilseed rape in Poland is exposed to *Leptosphaeria maculans* and *L. biglobosa*, which cause stem canker of *brassicas* (blackleg). They are responsible for considerable yield loss of oilseed rape. High profitability of oilseed rape production has caused great development of breeding varieties with new characters, including resistance to some diseases with different genetic backgrounds. Every year new population and hybrid varieties are introduced to the market. The aim of this study was to characterize the resistance of winter oilseed rape cultivars to phoma leaf spotting/stem canker in field conditions and to discriminate the species of *Leptosphaeria* by LAMP technique (Jedryczka et al. 2013).

**Material and Methods:** Screening of plant susceptibility/resistance was done in 2013 and 2014. The study aimed at comparing 13 (2013) to 44 (2014) cultivars of oilseed rape registered in Poland by the Central Station for Variety Testing (COBORU) or introduced to the candidate list due to high yield and the presence of *Rlm7* resistance gene. The experiment was done in Dlon (N51o41'22,0", E 17o04'23,0") Wielkopolska (Great Poland) region. The determination of *Leptosphaeria* species was studied using Loop-mediated DNA amplification (LAMP) method. For this purpose leaf and stem samples were collected from three individual plants per variety.

**Results:** In both seasons, the cultivars with *Rlm7* resistance gene showed significantly less symptoms, as compared to cultivars with no *Rlm7*. The resistance also differed significantly within cultivars with no Rlm gene. The isolates on leaves of cultivars without *Rlm7* resistance gene belonged mainly to *L. maculans* (72%), whereas on cultivars harbouring this gene they were scarce (5%) and all belonged to *L. biglobosa*.

**Conclusion:** The pathogen population of fungi causing blackleg of oilseed rape in Poland is still composed of *L. maculans* and *L. biglobosa*. The population of *L. maculans* is avirulent on Rlm7 resistance gene, i.e. contains *avrLm7* allele.

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**ORAL PRESENTATION THEME B**

# Suppression of clubroot of oilseed rape by soil amendments with different fertilizers

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**Background:** Clubroot, caused by *Plasmodiophora brassicae*, has been one of the most destructive diseases of oilseed rape in Germany and recently has become a more frequent problem worldwide. Our previous field studies showed the number of very virulent pathotypes has increased over the past years and subsequently commercial resistant cultivars become susceptible (Zamani Noor, unpublished data). Therefore, understanding the type of pathotype of the pathogen may be useful for developing better strategies to study the disease epidemiology, which should lead to more effective control of the disease.

**Objectives:** Previous studies described that when lime or calcium cyanamide are applied to the infected soil, the soil became suppressive to clubroot (Hwang et al. 2011; Dixon 2012) Field trials with natural infection on three different locations in Germany were conducted in 2014 to investigate control strategies for improving resistance in susceptible and resistant cultivars by evaluating the effect of different fertilizers application at different times during the growing season.

**Methods:** Calcium cyanamide (300kg/ha; 50% calcium oxide) and burnt lime (150kg/ha) were applied to the soil surface one day prior to the sowing or when the oilseed rape plants had reached the growth stage BBCH 11-12. Soil moisture, temperature and soil pH at two different depths (15 and 30 cm) were measured once every week after sowing date. Clubroot disease incidence and severity were assessed visually for the development of root galls.

**Results:** The preliminary results showed clear differences between the treatments. Changing the time of application had significant impact ($P \leq 0.05$) on the final severity of the disease. Relative to untreated control, clubroot incidence and severity were significantly lowered by application of fertilizer at later growth stages. In comparison with calcium cyanamide, burnt lime application has lower effect.

**Conclusions:** Clubroot is becoming to a major problem on oilseed rape fields in Germany. Preventing and controlling disease in contaminated fields is very difficult. Significant yield losses would result from disease development at early growth stages. At our study, application of calcium cyanamide after sowing has greatly reduced the disease severity and disease incidence.

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# Influence of soil moisture and leafhopper feeding densities on phytoplasma titres, aster yellow symptoms and seed yield of hybrid canola

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**Background:** Aster yellows (AY) caused major production losses to canola in western Canada in 2000, 2007 and 2012 (Olivier et al. 2011; Miller et al. 2013). The disease is caused by a phytoplasma vectored by the aster leafhopper, *Macrosteles quadrilineatus*. A five-point rating scale based on phyllody, virescence and presence of bladder-like pods was developed to assess the incidence and severity of AY symptoms in canola (Olivier et al., 2014). Symptoms appeared related to leafhopper densities and soil moisture.

**Objectives:** Investigate the effect of leafhopper feeding densities and soil moisture on the frequency and severity of AY symptoms and seed yield of hybrid canola.

**Methods:** Untreated hybrid canola was grown in dry soil (20-30% moisture content) and wet soil (70-100% moisture content). Plants at the early 2nd true-leaf stage were placed in cages (n = 4 plants/cage) and exposed to eight densities of AY-infected leafhoppers (n = 2 -16 adults/plant) for 10 hours. Numbers of leafhoppers feeding on each plant were recorded hourly. Plants were grown at 20°C under high light intensity (>400µmol/m2/s). The frequency and severity of AY symptoms were assessed after 6, 8 and 10 weeks using a five-point rating scale (Olivier et al., 2014). Droplet-digital PCR (Bahar et al., 2014) was done on the leaves, petioles, stems and roots of selected plants to quantify phytoplasma titres 8 weeks after infection. The remaining plants were grown to maturity and harvested to determine seed yield and 1000-seed weight. Analysis of variance and orthogonal contrasts for linear, quadratic and cubic trends were used to assess the effect of moisture and feeding densities on phytoplasma titres, AY symptoms, seed yield and 1000-seed weight.

**Results and Conclusions:** Soil moisture during infection had a pronounced effect on phytoplasma titres, incidence and severity of AY symptoms and subsequent seed yield of hybrid canola. Plants infected in dry soil had lower titres, fewer symptoms, less severe symptoms (AY rating 0-1) and higher seed yield than plants infected in wet soil. Titres, symptoms and yield in dry soil were not affected ($P \geq 0.05$) by leafhopper feeding densities. In contrast, phytoplasma titres and frequency/severity of AY symptoms in wet soil increased curvilinearly (linear and quadratic contrasts $P \leq 0.001$) as leafhopper feeding densities increased. The majority of plants exposed to feeding densities above 4 leafhoppers/plant had elevated phytoplasma titres in leaves, petioles and roots; severe AY symptoms (AY rating 3-5) and produced little or no seed. Relationships between feeding densities, phytoplasma levels, AY ratings and seed yield in dry and wet soil will be described.

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# Early fungicide application reduces blackleg impact on canola only when cultivar resistance is broken and the disease pressure is high

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**Background:** Although several fungicides are registered in Canada for control of blackleg [*Leptosphaeria maculans* (Desmaz.) Ces. & de Not] on canola *(Brassica napus)*, foliar fungicide application generally is not considered necessary in western Canada, especially on resistant cultivars (Kutcher et al. 2013). In recent years, blackleg has been increasing in much of the Canadian prairies, a circumstance attributable likely to shortened crop rotations and shifts in pathogen race structure. It is not clear, however, whether fungicide treatments should be recommended if cultivar resistance is overcome by the pathogen.

**Objectives:** To assess the benefit of fungicide treatments in relation to application timing and host resistance based on multi-site and multi-year field trials across canola growing regions in western Canada.

**Methods:** Field plots were established at five locations in western Canada between 2011 and 2012. The susceptible cv. Westar was used to represent the worst-case scenario of resistance breakdown. Diseased canola residues from previous years were left in the plot area for pathogen inoculum. The fungicides Headline® (pyraclostrobin), Tilt® (propiconazole), Quadris® (azoxystrobin) and Quilt Xcel® (propiconazole + azoxystrobin) were applied at the 2-4 leaf stage individually, in a split application (Headline then Tilt and vice versa) at the 2-4 leaf and prior to bolting, and Headline alone just prior to bolting. Unsprayed plots were used as a check. The resistant (R) cultivar 45H29 and moderately resistant (MR) cultivar 43E01 were treated with Headline at the 2-4 leaf stage only as additional checks. At crop maturity, blackleg incidence and severity were assessed on 50 plants by examining cross-sections of lower stems and tap roots in each plot. Seed yield was recorded after harvest.

**Results:** Data from a total of 17 site-years showed varying levels of blackleg. When all site-years were analyzed together, all treatments, except Tilt applied at the 2-4 leaf stage or Headline applied prior to bolting, reduced blackleg and increased seed yield of Westar. When data were analyzed separately based on disease severity (DS: 0-5), the trend was the same for trials with moderate to high levels (DS>1.0) of disease (8 site-years). However, no difference was observed with disease incidence, severity or seed yield under low levels (DS<1.0) of disease (9 site-years). Headline often reduced the disease incidence and severity on MR and R cultivars but did not increase the yield substantially.

**Conclusions:** Early application of pyraclostrobin or azoxystrobin reduced the impact of blackleg on canola when cultivar was lack resistance and the disease pressure was high. Foliar fungicide treatment for blackleg was of little benefit for MR and R cultivars.

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**ORAL PRESENTATION THEME B**

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# Integrated pest management of the rape winter stem weevil *(Ceutorhynchus picitarsis)* in France

**Background:** Rape winter stem weevil *(Ceutorhynchus picitarsis)* is a major pest for winter oilseed rape (WOSR) in France. Adults colonize fields in autumn to feed and lay eggs. Larvae are the harmful stage, since they grow in leaf stalks in winter and autumn and can migrate into the heart of the plant and destroy the terminal bud. Since 2009/2010, infestations have become very important in some French areas and despite repeated treatments, farmers are unable to control them. Several hypotheses can explain this situation, like re-infestation or resistant populations to pyrethroids.

**Objectives:** Since autumn 2011, CETIOM (The Technical Center for Oilseed Crops and Industrial Hemp) carried out studies to 1) understand the phenomenon, 2) develop new management strategies less dependent on insecticides.

**Methods:** For 3 years, CETIOM has monitored rape winter stem weevil autumn flights in untreated fields thanks to three kinds of traps. Coupled with this work, experiments were led to determine the more effective date of treatment, based on the egg laying dynamic. A monitoring was also performed to know if weevil populations were resistant to pyrethroids: insects were exposed in small bottles to several insecticide doses. Finally, we have been testing new strategies like associating frost-sensitive legume crops with WOSR or improving crop establishment. We also compared different varieties.

**Results:** In some experiments, we showed that there was little or no difference between plots, regardless of the date of treatment. Rape winter stem weevil arrivals were often spread out but this could not explain the lack of difference between plots in some experiments. The resistance monitoring showed that in these experiments, there were pyrethroid resistant rape winter stem weevil populations. Associating frost-sensitive legume crops with WOSR and improving crop establishment sometimes reduced weevil harmfulness.

**Conclusions:** In France, with the expansion of resistant populations to insecticides and the reduction of authorized chemicals, there is more than ever an urgent need to develop new management strategies which are less dependent on insecticides.

**Thanks:** Syngenta (financers), Axereal, Laboratoire d'Eco-entomologie for providing samples; J. Gauthier from FREDONCA; all CETIOM experimental stations and notably S. Clerget, R. Segura, Aurélie Ferraz, C. Pedebas, B. Auclert, N. Urvoit, A. Gremeret, D. Colin, F. Nourry; C. Pontet and D. Wagner for their help in data analysis.

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**ORAL PRESENTATION THEME B**

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# Impact of neonicotinoids suspension in EU28

**Background:** While the global number of managed honeybee colonies is increasing, there are still honeybee health challenges to solve. Research is under way to determine the cause of the Colony Collapse Disorder (CCD). The recent suspension of neonicotinoids in Europe in some crops attests to the growing concerns surrounding honey bee decline and the use of certain pesticides.

In 2012 a French-based study published in Science concluded that a small percentage of free-ranging honeybees whose brains were dosed with thiamethoxam at levels far in excess of field level doses became confused, failing to return to the hive.

The European Commission asked the European Food Safety Authority to study the safety of neonicotinoids. The study, published in January 2013, claimed that a high acute risk to honeybees was identified from exposure to neonicotinoids via dust drift from seed treatment uses in maize, oilseed rape and cereals. The study also claimed that an acute risk was identified from exposure via residues in nectar and/or pollen, but only at unrealistically high levels. On 29 April 2013, 15 of the 27 European Union Member States voted to restrict the use of imidacloprid, clothianidin and thiamethoxam for two years from 1 December 2013.

**The economic impact of neonics suspension in the European Union:** The Project Compass research report published by the Humboldt Forum for Food and Agriculture, an independent analysis, assessed the value of neonicotinoids in corn, sugar beet, oilseed rape, wheat, barley and sunflower across the EU economy. It was found that the neonicotinoids contribute to a total of 2.1 billion EUR p.a. to crop commodity market revenues, and that they improve production efficiency by 0.7 billion EUR p.a.

A partial suspension of neonicotinoids significantly reduces the competitiveness of EU production, reduces crop diversity and makes Europe more dependent on global imports of animal feed. The report forecasted that up to 50,000 full-time jobs could be lost, and growers would suffer an average income loss of up to about 5%.

Growers faced in the 2014 fall planting season challenges to establish the winter oilseed rape without neonicotinoids as seed treatment. In UK and Germany for instance, farmers had no choice but to return to older and less effective foliar insecticides to control important pests such as Cabbage stem flea beetles. The suspension will negatively impact the oilseed rape planted area due to the losses farmers face.

**Looking into the future:** The US Agriculture Department and the EPA concluded that neonicotinoids were significantly down the list of possible CCD contributors. They cited as primary drivers colony management, viruses, bacteria, poor nutrition, genetics and habitat loss. By far the biggest culprit—the report called it "the single most detrimental pest of honeybees"—was identified as the parasitic mite *Varroa destructor.*

# The effect of fungicides on *Leptosphaeria biglobosa* and *L. maculans*, phoma stem canker severity and oilseed rape yield

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**Background:** Phoma stem canker, a disease of oilseed rape *(Brassica napus)* caused by closely related pathogens *Leptosphaeria biglobosa* and *L. maculans*, is an economically important disease causing annual yield losses of approximately £1000M worldwide (Fitt et al., 2008). Both pathogens follow a monocylic disease cycle causing leaf spotting in autumn/winter and stem cankers in spring/summer. Severe cankers decrease transportation of water and nutrients to the developing seeds, resulting in reduced yield (Eckert et al., 2009). When colonising oilseed rape, *L. biglobosa* and *L. maculans* exist in close proximity on the leaf - competing for resources as they move down the main leaf vein and into the plant stem (Fitt et al., 2006). Fungicides are commonly used to decrease severity of phoma stem canker on oilseed rape. However, the efficacy and longevity of active chemicals is under threat from continuously evolving pathogen types (Carter et al., 2014).

**Objective:** To identify what effect commercially applied fungicides have on *L. biglobosa* and *L. maculans* interactions, phoma stem canker severity and oilseed rape yield

**Methods:** Winter oilseed rape field trials were done for three cropping season and phoma leaf spotting and phoma stem canker severity were assessed. Species composition in the stems of both upper stem lesions and basal stem cankers in the 2013/2014 cropping season was assessed using QPCR. The airspora for ispecies was monitored using a Burkard spore sampler, and species-specific DNA was quantified using QPCR. Fungicide sensitivity tests in vitro were done using fungicide amended agar plates at differing concentrations.

**Results:** The two pathogens differed greatly in their growth rates in vitro, with *L. biglobosa* growing much faster than *L. maculans*. The EC50 values show that *L. biglobosa* is significantly more tolerant of azole-amended media than *L. maculans*. Refinzar, containing active ingridents (a.i) penthiopyrad and picoxystorbin, was as effective at controlling phoma leaf spotting and phoma stem canker in the field as Proline (a.i. prothioconazole).

**Conclusions:** Differing sensitivities to azole fungicides could be selecting *L. biglobosa* resulting in an epidemic where current fungicides do not fully control the disease. A combination of SDHI + QoI fungicides could be used to control epidemics caused by a *L. biglobosa*.

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**ORAL PRESENTATION THEME B**

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# Pathogenic variability of *Sclerotinia sclerotiorum* isolates on *Brassica* differentials

**Background:** Sclerotinia rot (SR) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a major disease of oilseed *Brassica* all over the world. It is a ubiquitous, omnivorous, soil-borne phytopathogenic ascomycetes fungus capable of infecting more than 500 host species (Sharma et al. 2015). Infection occurs on leaves, stems and pods at different developmental stages, causing seed yield losses of up to 80%, as well as significant reductions in oil content and quality. Apart from favourable weather conditions and high soil moisture, germination of overwintered sclerotia, release, survival and germination of ascospores are important factors for the development of disease.

**Objectives:** Resistance in oilseed *Brassica* against the disease is lacking, only partial tolerance to *S. sclerotiorum* has been reported (Sharma et al. 2012). Keeping in view, the different *Brassica* differentials were challenged against 25 geographical isolates of *S. sclerotiorum* to confirm the variation among pathogen population and genetic difference in host species.

**Methods:** Twenty five geographical isolates of *S. sclerotiorum* causing SR of *Brassica* were collected during 2009-12 from 25 locations in 9 states of India and was maintained in vitro. An experiment was conducted to study the pathogenic variability during 2012-13 at ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, India (77°27 E, 27°12 N; 178.13 m MSL). Nine *Brassica* differentials i.e. *B. juncea* (cv. Rohini) *B. carinata* (cv. Kiran) *B. rapa* var *toria* (cv. PT 303), *B. rapa* var yellow sarson (cv. NRCYS 5-2), *B. rapa* var Brown sarson (cv. KOS 1), *B. nigra* (cv. BN-1), *B. napus* (cv. GSC6), *Eruca sativa* (cv. T-27) and *B. alba* were used during the study. These were sown in two replications and 65-70 days after sowing, plants were inoculated with stem inoculation technique. 3-weeks after inoculation the observations on stem lesion length and per cent disease incidence were recorded.

**Results:** All the 25 different geographical isolates showed significant variation in stem lesion length (cm) and per cent disease incidence. Based on pathogenic variability the isolates can be grouped as highly virulent (SR-06 and SR-10), virulent (SR-01, SR-02, SR-04, SR-08, SR-12, SR-15 and SR-25), moderately virulent (SR-03, SR-07, SR-09, SR-11, SR-17, SR-20, SR-21 and SR-24) and less virulent (SR-05, SR-13, SR-14, SR-18, SR-19, SR-22 and SR-23). Highly susceptible *Brassica* differential were all var of *B. rapa* and *E. sativa* while highly tolerant was *B. alba* (lesion size 0.5-1.9 cm).

**Conclusions:** Morphological variability and genetic diversity of different geographical isolates of *S. sclerotiorum* were already proved. The present study demonstrated existence of pathogenic variability among the geographical isolates which could be helpful to design resistance breeding for *S. sclerotiorum* in oilseed *Brassica*.

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# Screening of germplasm resources for resistance to *Plasmodiophora brassicae* in *Brassica napus L.*

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**Background:** Clubroot, induced by *Plasmodiophora brassicae*, endangers more than 100 species of plants, mainly in the tribe of *Brassicaceae*. The threat of clubroot has become more and more prominent to *Brassica* crops, including rapeseed *(Brassica napus L.)* and *Brassica* vegetables in China (Liang Y, et al.2001). In Sichuan Province, one of the biggest rapeseed producers in China clubroot is especially spreading rapidly in recent years. The occurrence of the disease has quickly expanded from the western plain areas to the central and eastern hilly areas in the province, causing an increasingly heavy loss of yield and quality in rapeseed. However, resistant rapeseed varieties are lack and other control practices could only exert limited effects on the disease (Liu Y et al. 2009). Breeding for resistant varieties to clubroot in *B. napus L.* is imperative.

**Objectives:** Screening of clubroot resistant materials from the existing rapeseed varieties and resource materials can be a rapid way to battle with the clubroot disease and will facilitate the breeding for resistant varieties in *B. napus L.* (Liu Y et al. 2009; Fu M L et al. 2011). The major objectives of the present study are to know the status of resistant materials in our own resource breeding materials and to screen out clubroot-resistant materials for breeding of clubroot resistant varieties.

**Methods:** A total of 279 lines of *B. napus L.* from our breeding stock were selected to identify the resistance to clubroot *(P. brassicae)*, including 160 pure lines and varieties collected from rapeseed institutions in China, 93 elite inbred lines and 26 resynthesized (RS) lines developed in our own Rapeseed Research Center. Spores of *P. brassicae* were isolated from root tumors of infected rapeseed plants *(B. napus L.)* collected from the severely infested rapeseed fields in Dayi county on the plain area of Sichuan Province. The suspension of spores was adjusted to a density of 1 x108 / ml. The inoculation to the rapeseed seedlings was made at the 2-true-leaf stage in greenhouse. The identification of disease incidence and severity index in the tested lines was done for 20 to 30 plants in each line, 50 days after inoculation. In order to confirm the results from the first primary tests, 54 selected lines comprising relatively highly resistant, moderately resistant, slightly resistant and susceptible lines were tested again with three replications by the same procedure. Classification of resistance grades of the tested lines was evaluated with a five-grade standard based on the disease severity indices: highly resistant (<30.0), moderately resistant (30 ~ 40), slightly resistant (40.0 ~ 50.0), susceptive (50.0 ~ 80.0), highly susceptive (> 80.0).

**Results:** In all the 279 lines of the first primary test, only one line (H484-2, RS line) was found to be very low in disease incidence (16.67%). The all others showed a high incidence ranging 85 to 100%. Ninety-seven percent of the tested lines showed an incidence of above 90%. The severity indices of all the lines ranged from 7.14 and 97.28. Only 2 lines (H484-2, RS line and D4TZ, progeny of German line X RS line) showed a low severity index of < 30.0, grouped in the highly-resistant category. Seven lines, including 2 canola-quality lines, showed relatively low severity indices between 40.0 and 50, grouped in the slightly resistant category. All the others showed high severity indices larger than 50.

In the 54 lines for the second test, the average incidences of infected plants ranged from 9.83% to 100%. About 57% of the lines showed an incidence of over 80%. The severity indices were between 4.66 and 79.88. Six lines were evaluated in the highly resistant category, 4 lines in the moderately resistant category, and 26 lines in the slightly resistant category. Summarizing the two consecutive experiments, only two lines (H484-2, D4TZ) were confirmed to be consistently highly resistant, and 2 lines (H218-4, NLS-1) to be consistently slightly resistant to clubroot.

**Conclusions:** Based on the two tests, we concluded that in our resource materials of *B. napus L.*, highly resistant materials to clubroot disease are in a dramatic shortage. Only 2 lines were confirmed to be highly resistant and 2 confirmed to be slightly resistant. Meanwhile, the occurrence and severity of clubroot disease are affected by many factors. All the lines should be further tested with different pathotypes of *P. brassicae* and under strictly controlled conditions. Especially, the materials with a severity index of less than 50 must be evaluated again, to procure the reliable highly resistant lines.

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**ORAL PRESENTATION THEME B**

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# Use of the effector-triggered defence concept to identify candidate resistance genes

**Background:** Effector-triggered defence (ETD) is a plant response against invading apoplastic fungal pathogens (Stotz et al., 2014). This defence response depends on recognition of apoplastic effectors by receptor-like proteins (RLPs). Based on the assumption that RLPs play a critical role in defence against apoplastic pathogens, new opportunities for disease resistance breeding have emerged. Oilseed rape is attacked by apoplastic pathogens, including *Leptosphaeria maculans* and *Pyrenopeziza brassicae*, which cause phoma stem canker and light leaf spot, respectively. The first RLPs cloned from oilseed rape operate against *L. maculans*.

**Objectives:** The main objective is to define the RLP complement of *Brassica napus* from its recently published genome. The distribution of RLP genes and other aspects of genomic information are used to determine their usefulness in predicting resistance genes operating against apoplastic pathogens of oilseed rape.

**Methods:** A protein motif-based search was used to define the RLP complement in *B. napus*. This information has been used to visualize all predicted RLPs on the 19 chromosomes of *B. napus*. This data will be statistically analysed to develop a tool to assist in resistance gene identification. To understand physical interactions between leucine-rich repeat (LRR) domains of resistance (R) proteins and corresponding effectors that are directly recognized by host receptors, computational molecular docking was used. Structures of complexes were predicted from the known structures of effectors and models of LRR domains. This will be relevant for interactions between *Rlm2* and *AvrLm2* and *Rlm4* and *AvrLm4*.

**Results:** The distribution of RLPs across the *B. napus* genome has been visualized. Like nucleotide-binding LRR receptors (NLRs) that operate against haustoria-forming filamentous pathogens, RLPs are clustered. The distributions of NLRs and RLPs differ in oilseed rape.

**Conclusions:** Based on the genomic analyses of RLPs, new tools will become available to accelerate cloning of R genes that control ETD. Genome-guided methods have already been used to identify R genes that encode cytoplasmic NLRs; these are important for effector-triggered immunity (ETI) against haustorial pathogens. More detailed information on specific amino acids in R proteins that interact with pathogen effectors can be derived from docking of interacting proteins.

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# A hidden pathogen with uncommon properties – reviewing the state-of-the-art of *Verticillium longisporum* on oilseed rape

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**Background:** *Verticillium longisporum* (VL) is a soilborne vascular pathogen of oilseed rape (OSR; *Brassica napus)* which causes premature ripening associated with potential significant yield losses. First reports on the disease date back to the 1960s in Sweden and the 1980s in North-East Germany, but more recently outbreaks of the disease were observed in the UK, France and Poland. In 2014, occurrence of VL has been confirmed for the first time in Canada (Manitoba). The pathogen is an amphihaploid hybrid of *Verticillium* and host-specific on B*rassicaceae*.

**Objective:** This research aims at elucidating the interaction of VL with oilseed rape, particularly focusing on differential resistance responses in *B. napus* lines and the damage potential of the disease in the field.

**Methods:** *B. napus* accessions and their parental lines have been screened in the greenhouse and field. Interactions on plants inoculated in the climate chamber or laboratory were studied on the phenotypic, histological (CLSM), transcriptomic and metabolomic (physiological and biochemical) level.

**Results:** Three different hybrids representing three genetically distinct lineages have been identified so far and were shown to differ in host specificity (Novakazi et al. 2015). Hyphae of VL attach to the roots in the root hair zone and penetrate the rhizodermis to colonize the root parenchyma intra- and intercellularly (Eynck et al. 2007). The lack of ROS generation or cell death responses by the plant during these early stages of interaction implies a biotrophic or even endophytic relationship. Following root invasion, the fungus remains strictly xylem-limited and colonizes the hypocotyl, where intense plant responses are triggered as expression of cultivar resistance (Eynck et al. 2009). Resistance derives from the C-genome of *B. napus* (AACC). Cultivar resistance is expressed in the hypocotyl and consists of fast and massive parenchymatic accumulation of cell wall-bound phenols, lignin and vessel occlusions, obviously halting the pathogen from further spread into the shoot. No drought stress parameters (stomatal conductance, transpiration rate, gas exchange, photosynthesis rate, proline content, leaf water content) are induced under VL infection. VL induces salicylic acid (SA) dependent genes PR-1 and PR-3 while the jasmonic acid/ethylene dependent PDF1.2 does not respond. VL induces strongly elevated levels of SA/SAG in xylem sap and stem parenchyma, which surprisingly correlate with susceptibility and disease severity. Increased levels of SA/SAG in diseased plants may indicate a fungus-induced rerouting of precursors from the phenylalanine/cinnamate pool towards SA synthesis depriving synthesis of CW-bound phenolics crucial for resistance. In contrast, SA deficient nahG transformed OSR plants exhibit a strongly elevated susceptibility to VL.

**Conclusions:** The findings so far demonstrate that VL is an atypical vascular pathogen in not inducing any wilt in its host. It is host-specific to *Brassicaceae* but may expand its host range by forming diverse pathotypes. Cultivar-specific resistance derives from the C-genome, is partial and quantitative, halting the fungus only at the hypocotyl interface. Resistance is dependent on CW-bound phenolics and lignin. A dual role of SA exists in basal and cultivar-specific resistance of *B. napus* to VL.

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**ORAL PRESENTATION THEME B**

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# DNA-based soil test a prerequisite for Swedish oilseed rape production

**Background:** Clubroot caused by *Plasmodiophora brassicae*, is recognised as a serious soil-borne disease in *Brassica* crops associated with appreciable yield losses. Disease outbreaks have in recent years caused problems in Sweden and world- wide in oilseed rape (OSR). Resistant cultivars of winter oilseed rape (WOR) are now available for the growers. Fast and reliable detection methods are a prerequisite for prediction of the infection potential of clubroot in field soils for implementing integrated management strategies in OSR production and crop development.

**Objectives:** Development and validation of a *P. brassicae* -specific real-time PCR assay, and to use this assay as a quantitative measure for direct detection of *P. brassicae* in naturally infested soil samples. Further, to combine these quantitative measures with methods for spatial prediction to assess the risk of disease development within fields.

**Methods:** A procedure using real-time PCR for the direct detection and quantification of *P. brassicae* in soil samples was developed and used for naturally and artificially infested soil samples containing different concentrations of *P. brassicae*. Bait-plants were used to validate the real-time PCR assay. The spatial distribution of *P. brassicae* DNA was determined and indicator kriging was used for spatial prediction to assess the probability of disease development within fields.

**Results:** Species-specific primers and a TaqMan fluorogenic probe were designed to amplify a small region of *P. brassicae* ribosomal DNA. The PCR assay was optimised to give high amplification efficiency and 3-4 copies of the target DNA sequence were detected. The inter-sample reproducibility was similar to, or higher than, that of assays for other pathogens quantified in soil samples. The detection limit in soil samples corresponded to 500 resting spores g-1 soil and the estimated probability of clubroot based on an increased sampling density (n=40) showed an overall spatial trend in the variation of the amount of *P. brassicae* inoculums.

**Conclusions:** The integration of resistance as a management tool, along with other control measures, offers a robust management strategy. Soil tests are a prerequisite for growers and for successful field experimental activities. This rapid and sensible assay for predicting infection potential and distribution within fields for routine risk assessment is now commercially available in Sweden. The assay is used as guidance prior to seeding susceptible crops and as a routine for the Swedish Seed and Oilseed Growers Association for determining field experimental sites in farm fields. Data for interpreting soil tests are currently based on results from field tests of partly resistant cultivars of summer oilseed turnip rape. Activities are ongoing to carefully follow the reactions of resistant cultivars of winter oilseed rape at field level. Complementary knowledge of the prevalent pathotypes is required, since tolerance in resistant cultivars is observed and seems to vary in response to different pathotypes of clubroot.

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# Overexpression polygalacturonase-inhibiting protein 2 of rice inhibits *Sclerotinia sclerotiorum* polygalacturonase necrotizing activities and improves resistance in transgenic *Brassica napus*

**Backgroud:** Canola *(Brassica napus L.)* is an agriculturally and economically important crop in China, and its growth and yield are influenced by fungal pathogens. *Sclerotinia sclerotiorum* is a necrotrophic plant pathogen that causes stem rot disease in *B. napus*. *S.sclerotiorum* secretes several types of pectin-degrading enzymes, including pectin methylesterases, pectin lyases, and polygalacturonases (PGs). Polygalacturonase-inhibiting proteins (PGIPs) are typically leucine-rich repeat (LRR) proteins counteracted the action of PGs. PGIPs prevent cell wall degradation, therefore hamper the invasion process and the release of nutrients that is necessary for pathogen growth. Overexpression of *Atpgip1* and *Atpgip2* in *Arabidopsis* limits the colonization by *B.cinerea* and reduces disease symptoms. Overexpressing a pear pgip can increase PG-inhibitory activity and a decrease in susceptibility to *B. cinerea* in transgenic tomato and grapevine plants (Di Matteo A et al. 2006). In rice, *OsPGIP* was able to inhibit the PG of Fusarium graminearum (Lu et al. 2012). These findings suggest PGIPs are important players in plant innate immunity.

**Objective:** Overexpression of *OsPGIP2* in partial resistant *B. napus* 7-5, T45 and susceptible P61-5 material will improve resistance to *S. sclerotiorum*. We hope to discover the molecular mechanism of *OsPGIP2* interaction with *S. sclerotiorum* PGs (SsPGs).

**Methods:** *S. sclerotiorum* was cultured at 20 °C on potato dextrose agar. Mycelia plugs (8 mm diameter), excised from the edge of actively growing colonies and inoculated onto detached leaves and stem. Expression of SsPGs and *OsPGIP2* were induced by *Pichia pastoris* system. ROIs (Reactive Oxygen Intermediates) production and cell death were determined by DAB and Evans blue staining

**Results:** The *OsPGIP2* overexpressed lines of three different materials exhibit a delay in the onset of symptoms upon *S. sclerotiorum* inoculation in seedling stage and adult stage, compared to their individual untranformed plants (WT). DAB and Evans blue staining suggest *OsPGIP2* lines have lower ROIs and less cell death than their WTs. *Pichia pastoris* system and in vitro analysis indicate *OsPGIP2* inhibits SsPG6 enzymatic activity. Defensive related genes involved in jasmonic acid and ethylene (JA/ET) or salicylic acid (SA) signaling pathway expression analysis with inoculation reveals that six genes *(BnCCR 'BnOPR 'BnLectin 'BnWRKY6 'BnSOT and BnPDF1.2)* show enhanced expression in transgenic lines derived from three individual materials. JA/ET signaling pathway in *OsPGIP2* transgenic lines is obviously activated by *S. sclerotiorum*.

**Conclusions:** The molecular mechanism of interaction between *PG-OsPGIP2* could be a help in understanding *OsPGIP2* can improve the resistance to *S. sclerotiorum*. Field protection assay should be estimated for transgenic lines derived from three materials. We will focus on the resistance pathways initiated with *OsPGIP2* concerning *Brassica napus* PGIPs.

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**ORAL PRESENTATION THEME B**

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# Efficiency of major gene mediated resistance in *Brassica napus* to *Leptosphaeria maculans* in different regions of Germany

**Background:** Blackleg disease, caused by *Leptosphaeria maculans* (LM) is one of the most important fungal diseases in oilseed rape (OSR) production word-wide (Fitt et al. 2006). Genetic resistance is an important tool to control this disease. Seedling resistance is conferred by single major genes. Due to its sexual propagation, LM isolates evolving rapidly from avirulent to virulent strains on cultivars harboring major resistance genes. Therefore, resistance of OSR against LM conferred only by major resistance genes was often overcome and led to severe yield losses in the past (Rouxel et al. 2003; Sprague et al. 2006).

**Objectives:** The aim of this study was to determine the efficiency of major resistance genes to LM in different OSR growing regions in Germany by identifying the frequency of virulent isolates and determining the race spectra of LM.

**Methods:** We cultivated two OSR cultivars in fields throughout Germany from 2011 to 2014: i) 'NK Bravour' harboring no known major genes against LM (serving as trap crop) and ii) 'Exocet' harboring the efficient major gene *Rlm7* to observe resistance breakage in the field. In autumn and spring we collected true leaves with typical Phoma lesions to gain isolates of LM. Single pycnidia isolates were tested with a French and Canadian differential set through cotyledon inoculation for their virulence to different major genes. The differential set consisted of 10 OSR genotypes harboring the major genes *Rlm1, Rlm2, Rlm3, Rlm4, Rlm7, Rlm9* and *LepR1, LepR2* and *LepR3*. Thereby, the frequency of virulent isolates in a region was determined. Isolates showing the same complement with virulence alleles were grouped to the same race.

**Results:** The frequency of isolates being virulent to *Rlm1, Rlm2, Rlm3, Rlm4* and *Rlm9*, respectively, was above 85%. Conversely, the frequency of virulent isolates to Rlm7 was very low (< 5%). Interestingly, the frequencies of isolates being virulent to the major genes *LepR2* and *LepR3* showed a high variability between different regions, ranging from 35% to 100%. There was no isolate showing virulence to LepR1. Most isolates belonged to two races with a high virulence complexity.

**Conclusions:** Most tested major genes lost efficiency to LM. Only *Rlm7* and *LepR3* are still mediating resistance in OSR to PM in Germany. We assume that *Rlm7* may lose its efficiency with increasing deployment of this major gene in OSR in Germany.

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**ORAL PRESENTATION THEME B**

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# Mapping and SNP marker development in *Brassica* diploid species for breeding canola and mustard resistant to clubroot

**Background:** Clubroot disease, caused by *Plasmodiophora brassicae*, is an ever increasing problem in canola and mustard production in western Canada. A number of clubroot resistant (CR) canola *(Brassica napus)* hybrids are now available in Canada. However, there is some urgency about the erosion of CR in these hybrids with the emergence of a new pathotype. Identification of novel CR genes and pyramiding of these genes is the most effective approach to develop varieties with durable and broad spectrum resistance. However, the sources of resistance to clubroot in *B. napus* are very limited and no resistance was found in the mustard species *B. juncea* and *B. carinata*. Resistant germplasms highly resistant to clubroot were identified in the diploid *Brassica* species *B. rapa, B. oleracea* and *B. nigra* (Peng et al. 2014).

**Objectives:** Map CR genes from newly identified CR in diploid species using next generation sequencing technologies; develop SNP markers tightly linked to CR genes and transfer CR genes into canola *B. napus* and mustards *B. juncea* and *B. carinata*.

**Methods:** Segregating populations were made by crossing CR lines with susceptible lines. DNA sequencing or RNA-sequencing was performed with Illumina HiSeq or MiSeq platforms. Validation and genotyping of SNPs were carried out using the KASP method. Introgression of resistance to the amphidiploids was carried out using conventional breeding and molecular genetic approaches.

**Results:** Identification of CR genes was carried out in five *B. rapa* cultivars/breeding lines and five CR genes *Rcr1* to *Rcr5* were fine mapped. *Rcr1* was identified from bok choy, *Rcr2* from Chinese cabbage, *Rcr3* and *Rcr4* from canola and *Rcr5* from turnip. Four genes *(Rcr1, Rcr2, Rcr4* and *Rcr5*) were identified on *B. rapa* chromosome A03 and one (Rcr3) on A08. More than 30 robust SNP markers associated with the CR genes on A03 and four with the CR gene on A08 were developed. CR gene *Rcr6* was identified in *B. nigra* and mapped into *B. nigra* chromosome B3 corresponding to a region in *B. rapa* chromosome A08. In *B. oleracea*, one gene Rcr7 was mapped on chromosome C7. Interspecific crosses with a *B. napus* canola line were performed using the CR donors in the diploid species and then back crossed with the *B. napus* canola line. Resistance to clubroot was confirmed in the introgressed *B. napus* lines by evaluation for CR and marker assisted selection. Amphidiploid lines in *B. napus, B. juncea* and *B. carinata* resistant to clubroot were resynthesized using CR lines from the respective diploid species.

**Conclusions:** Seven CR genes were mapped in all three *Brassica* diploid species and sexually transferred to the amphidiploids. Robust SNP markers tightly linked to each gene are available for marker assisted selection in canola and mustard breeding programs.

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