Characterization of candidate genes within QTL associated with resistance to stem rot in a doubled haploid breeding population of canola

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Background: Stem rot in canola (*Brassica napus*) is a fungal disease caused by *Sclerotinia sclerotiorum*. Resistance to this economically important disease in canola is considered a quantitative trait. Genotyping-by-sequencing of an F_2 population derived from a cross between doubled haploid parents susceptible (NEP32) or resistant (NEP63) to stem rot identified >20,000 polymorphic SNP markers between the two parents. These markers were used to construct a genetic map with 1847 marker loci with an average marker density of 8 cM per marker that corresponds to 0.37 Mb of the genome. Composite interval mapping identified five significant QTL on chromosomes A01, A03, C01 and C08 associated with resistance to stem rot that account for 10 to 19% of the phenotypic variation (Chittem et al. 2015a). RNAseq data obtained from the two parents (NEP32 and NEP63) in response to inoculation with *S. sclerotiorum* identified several differentially-expressed genes within each of the five QTL associated with resistance to stem rot (Chittem et al. 2015b).

Objectives: Determine if differentially-expressed genes identified in the parental germplasm and that fall within the five QTL associated with resistance to stem rot correlate to resistance or susceptibility of the F_2 progeny.

Methods: Sequence obtained from the parental lines (Chittem et al. 2015b) and the draft genome of canola (Chalhoub et al. 2014) were used to identify candidate genes within the five QTL associated with stem rot resistance. Primer pairs to at least one gene showing significant differential expression among the five QTL were designed using the Primer Select program of DNASTAR Lasergene 8 software. A subset of canola plants with varying levels of stem rot resistance within the F_2 progeny were inoculated with or without S. sclerotiorum (NE 152). RNA extraction, cDNA synthesis, and qRT-PCR were conducted as previously described by Doğramacı et al. (2014).

Results: Candidate resistance genes within the five QTL associated with stem rot resistance are being characterized to determine if their expression levels correlate to differing degrees of resistance in the F_2 breeding population. Candidate genes being tested for correlation to stem rot resistance in this study include: BnaA01g14790D (putative nucleotide hydrolase), BnaA03g36620D (putative alternative oxidase), BnaC01g24700D (putative Myb-like transcription factor), BnaC08g37640D (putative CCAAT-binding transcription factor), and a novel transcript of chromosome C01 (putative ethylene-responsive transcription factor).

Conclusions: will be based on outcome of results.

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315
POSTERS THEME B

Analysis of the interaction between the avirulence genes *AvrLm4-7* and *AvrLm3* in *Leptosphaeria maculans*

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Background: *Leptosphaeria maculans* is the fungus responsible for phoma stem canker (blackleg), a damaging disease on canola (*Brassica napus*). The deployment of resistant *B. napus* cultivars is known to be the most effective way to control this disease. Specific resistance genes (*Rlm*) are efficient whenever the corresponding avirulence (*AvrLm*) allele is prevalent in fungal populations. A complex relationship has been identified between two avirulence genes of *L. maculans*: *AvrLm3* and *AvrLm4-7*. When an isolate possesses both avirulence genes, only the avirulence towards *Rlm7* is expressed. The *AvrLm3* avirulence phenotype is only expressed when an isolate displays a deleted or a non-functional allele of *AvrLm4-7*.

Objectives: Following the cloning of *AvrLm4-7* (1), we cloned *AvrLm3* to decipher the functional relationships between *AvrLm3* and *AvrLm7* and to understand the role of each avirulence protein in pathogenicity.

Methods: An *AvrLm3* candidate gene was identified combining several approaches: genetic mapping, BAC-clone sequencing and RNA-seq of plant-pathogen interaction. Complementation and silencing assays were performed to validate the *AvrLm3* candidate and the functional antagonism between *AvrLm3* and *AvrLm7* phenotypes. The presence of *AvrLm3* and its allelic diversity were analyzed in a large collection of world-wide isolates.

Results: The *AvrLm3* locus is genetically close to *AvrLm4-7* and located in a genomic region partially absent from the assemblies of the reference sequence genome of *L. maculans* as well as from a series of resequenced *L. maculans* genomes. *AvrLm3* encodes for a small secreted protein, rich in cystein residues and the gene is highly expressed at early infection stages. The genotyping of field isolates showed that *AvrLm3* is highly conserved in field populations, with no isolates displaying deletion of *AvrLm3*. Only one avirulent allele but several virulent alleles have been identified. Complementation of an avirulent isolate towards *Rlm3* with a functional allele of *AvrLm4-7* leads to avirulence towards *Rlm7* and to the loss of the avirulence towards *Rlm3*.

Conclusions: We cloned a new avirulence gene of *L. maculans* which has classical *AvrLm* genes characteristics. *AvrLm3* is involved in a complex interplay with *AvrLm4-7*. A similar interaction has only been identified in one other ascomycete, *Fusarium oxysporum* (2). In the context of the outbreak of isolates virulent towards *Rlm7* due to the massive deployment of *Rlm7, Rlm3* is becoming effective again. Therefore, this negative interaction between two avirulence genes offers opportunities for the development of strategies for sustainable management of resistance genes. A modelling approach is in progress to determine which strategy, among pyramiding or alternating *Rlm3* and *Rlm7*, is the best to be deployed.

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Identification of cotyledonary resistance to *Leptosphaeria maculans* (the causative agent of blackleg) in cultivated *Brassica oleracea* accessions

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Background: The genetic resistance to blackleg in canola cultivars can be easily eroded by the changes in the virulence of field populations of the causative agent *Leptosphaeria maculans*. Breakdown of the host resistance has been reported in several countries in Europe and in Australia (Rouxel et al. 2003, Li et al. 2003).

Objective: In Australia, the major genes *Rlm2, Rlm9, LepR3* have broken down and there are no longer effective for breeding purposes. As a result, there is a constant challenge to discover novel sources of blackleg resistance in related plant species. To date, major resistance genes to blackleg have been discovered only in the A genomes (*Brassica rapa* and *B. napus*) and B genomes (*B. carinata* and *B. juncea*) with none reported in the C-genome.

Method: For this study, 37 cultivated *B. oleracea* accessions were screened in controlled environment conditions. The gene profile was identified using a differential set of 12 field-derived single-spore isolates of *L. maculans* (Marcroft et al. 2012). The experimental design was randomised block design with 30% partial replication. Cotyledons of 10 days old seedlings were wounded and inoculated with 10 µL of each of the individual isolates. Lesion scores were determined 14 to 21 days after inoculation.

Results: Three cultivated *B. oleracea* accessions have shown high levels of cotyledonary resistance to a number of the differential isolates.

Conclusion: This paper reports the identification of resistance to blackleg in the C-genome of diploid *Brassica oleracea* accessions, for the first time. These resistant lines will be utilised in a re-synthesis program and molecular markers for these genes will be sought.

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317
POSTERS THEME B

Standing up against a bully: Plant defense mechanisms underlying blackleg resistance in *Brassica napus*

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Background: *Leptosphaeria maculans* (Blackleg) is a devastating fungal pathogen of *Brassica napus* (canola) and causes millions of dollars in crop damage and loss to growers around the globe each year. Current blackleg disease management strategies rely heavily on gene-for-gene resistance, however the genetic mechanisms mediating these interactions in canola remain poorly characterized.

Objectives: We are interested in understanding defense strategies against the devastating fungal pathogen, blackleg in canola at the molecular level using cutting edge transcriptomics. We studied the transcriptome in a commercially available resistant and susceptible line before, during and after blackleg inoculation of the cotyledons to identify the genes and gene regulatory processes associated with plant resistance.

Methods: We sequenced canola cotyledons in Westar (susceptible) and DL15 (*LepR1*) before, during and after the infection processes at the level of the RNA. A total of 36 RNA sequencing libraries were analyzed using robust bioinformatics strategies to identify differentially expressed genes between control and infected tissues. Fuzzy K-means clustering and GO term enrichment analyses revealed dominant patterns of genes activity thought to control cellular defense mechanisms. We complemented our transcriptome series with a comprehensive histological analysis at the light and electron levels.

Results: Both susceptible and resistant cultivars had an increased abundance of transcripts associated with canonical defense responses like jasmonic acid, ethylene, and salicylic acid signaling, production of antifungal compounds and phytoalexins, and programmed cell death. Populations of transcripts were found to accumulate specifically in the *LepR1*-mediated resistant cultivar. This included a variety of highly active kinases and other transcripts that code for proteins that have demonstrated the ability to carry out the oxidative burst. In addition, transcripts coding for transcription factors, calmodulin binding proteins, and other upstream signaling proteins were found to be up-regulated only in the *LepR1* containing cultivar.

Conclusions: We provide the most comprehensive blackleg infection dataset produced to date in the cotyledon of *B. napus*. A subset of transcripts were found to accumulate specifically within the *LepR1* resistant cultivar during infection and may coordinate the defense response necessary for plant resistance at the seedling stage of development. These data provide an informatics resource for those interested in the genes responsible for mediating blackleg resistance in canola.

318
POSTERS THEME B

Optimal agronomic conditions for spring and winter canola production in northern Idaho

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Background: Lack of economically viable alternative crops to grow in rotation with small grain cereals has increased producers interest in growing spring and winter canola. Higher yielding canola cultivars combined with competitive prices has resulted in an increase of canola acreage in the Pacific Northwest region. Although adapted canola cultivars are now available to growers, few attempts have been made to optimize productivity through agronomic management of the crop.

Objectives: The aim of this two-year study is to optimize growers' productivity and profitability with a range of adapted winter and spring canola cultivars in specific environments. Agronomic factors examined include planting date, seeding rate, and fertility management in two different tillage systems.

Methods: Two winter canola cultivars were planted at two locations in July and in August in the fall of 2013 and 2014. Similarly, four spring canola cultivars were planted at two locations as early as possible and two weeks later in the spring of 2013 and 2014. At each planting, cultivars were seeded at three rates and were grown with six nitrogen levels in replicated yield trials. A range of morphological characters were recorded throughout the growing season. At harvest seed yield and oil content was recoded.

Results: Amanda was the highest yielding winter cultivar (3,582 kg ha-1) over all factors examined and responded significantly better to increased nitrogen levels compared to HyClass-125 (3,168 kg ha-1). Early planting winter canola produced higher seed yield (3,517 kg ha-1) compared to later plantings (3,235 kg ha-1), and yield from intermediate and high seeding rates were not different. Interactions between spring cultivars and most agronomic factors were often significant but small in magnitude compared to the main effects. All the spring canola cultivars produced higher yields when planted early, at intermediate seeding rates and with moderate to high nitrogen availability; although, as with the winter cultivars, the spring cultivars responded differently to nitrogen availability. Highest spring yield was 2,684 kg ha-1 for the cultivar DKL 30-42 with 249 kg N ha-1. Agronomic factors had little effect on seed oil content for either winter or spring cultivars tested.

Conclusions: In general early planting was advantageous for both winter and spring canola cultivars. Low seeding rates can reduce yield potential in most but not all situations. Cultivar choice has the largest impact on overall yield potential. However, cultivars did interact with regard to nitrogen application and it is critical to determine optimal nitrogen requirements on a cultivar by cultivar basis.

319
POSTERS THEME B

On-farm studies of canola insect pests: effects of insecticide application and seeding date on seedpod weevils, lygus and seed yield

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Background: Cabbage seedpod weevil is a chronic exotic pest of Canola in southern Alberta and Saskatchewan. The main damage is caused by larvae feeding on seeds, therefore, growers spray insecticide at early flower to target adults. Lygus bugs, on the other hand, are native pests that attack many crops including canola and are controlled, also with insecticides but sprayed later at the pod stage. In the drier Short Grassland Eco-Regions of Alberta the dominant species are *L. elisus* and *L. borealis* and *L. keltoni* (Carcamo et al. 2002). Lygus bugs can increase rapidly under dry hot weather through reproduction and dispersal from other sites as plants senesce (Otani and Carcamo 2011)

Objectives: Determine impact of spraying insecticide for cabbage seedpod weevil at early flower on abundance of lygus bugs at early pod in commercial farms and the effect of seeding date on the management of this pest complex.

Methods: Insects were sampled using a sweep net in 75 farms from 2010 to 2013. Four to eight strips (about 25 m wide and 50-100 m long) along the borders of the fields were left unsprayed to assess the impact of the insecticide on insects and seed production. Yield was collected from all these sites using 64 quadrat samples (0.25 m2) per site. For 20 fields of these fields we also obtained the yield from the farmers combine monitors. Also, damage to pods by seedpod weevil was determined from sub-samples from the main racemes of selected fields. For all fields we obtained information on seeding dates and cultivars seeded and local rainfall for selected fields.

Results: Early seeding in April increased the risk of having high weevil pests but decreased the risk of lygus. Sites planted latest, during the last two weeks of May, had the most lygus and the fewest weevils and those planted at a normal period (first 2 weeks of May) had intermediate numbers of both pests. Spraying insecticide at early flower resulted in fewer lygus at the pod stage in most fields and a yield increase over the untreated strips of approximately 100 kg/ha (2 bu/ac) across all sites. However, there was very high variability from site to site and year to year. Correlation analysis suggested that lygus bugs at early flower were not related to yield or to lygus numbers at later crop stages.

Conclusions: Growers should not extrapolate economic thresholds for lygus below one per sweep even at high canola prices. Canola fields planted early with less than 2-3 weevils per sweep should not be sprayed prophylactically for lygus bugs.

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Mapping and identification of clubroot resistance genes in *Brassica nigra*

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Background: Clubroot disease caused by *Plasmodiophora brassicae* is one of the most serious diseases that affect the plant family *Brassicaceae*. It is an emerging threat to canola and mustard production in western Canada. To manage the disease, it is very important to identify and use new sources of clubroot resistance (CR) for developing canola and mustard cultivars. *Brassica nigra* lines with a broad spectrum of resistance to clubroot were recently identified (Peng et al. 2014).

Objectives: The project aims to identify CR genes and fine map the genes in *B. nigra* for facilitating map-based gene cloning; and to develop robust genetic markers tightly linked to the CR genes for use in marker-assisted selection in canola and mustard breeding programs.

Methods: Plant materials were as follows: resistant (R) lines BRA and PI; susceptible (S) line CR2748. The two CR *B. nigra* lines were crossed with CR2748, respectively to produce the F1. F1 plants were self-pollinated to produce F2. F1 plants from CR2748 X PI and CR2748 X BRA were backcrossed with CR2748 plants to produce BC1 populations. Genetic mapping of CR genes was carried out using bulked segregant RNA-Seq described by Liu et al (2012). RNA was isolated from each bulk using RNeasy Plant Mini Kit (Qiagen). The cDNA libraries were prepared using TruSeq RNA Sample Preparation Kits v2 (Illumina). RNA-seq was carried out on MiSeq platform. Validation and genotyping of SNP markers were carried out using Kompetitive Allele Specific PCR method.

Results: Complete resistance to clubroot was found in all F1 plants derived from crosses of CR2748 with PI and BRA, respectively. Evaluation for resistance to clubroot showed ratios of 1R:1S in BC1 and 3R:1S in F2 for both R genotypes, indicating that CR is controlled by a single dominant gene in either PI or BRA. Short reads from R and S bulked RNA-seq samples in the BC1 population derived from PI were assembled into the *B. rapa* reference genome v1.5 respectively. A CR gene designated Rcr6 in PI was mapped in a region homologous to *B. rapa* chromosome A08. Three SNP markers (SNP_A08_14, SNP_A08_15 and SNP_A08_17) linked to Rcr6 were developed. Plants in the BC1 population with BRA were also analyzed with the SNP markers linked to Rcr6 and results showed that resistance in BRA was not associated with the Rcr6 linked SNP markers, indicating that the CR gene namely Rcr8 in BRA is not in the Rcr6 region.

Conclusions: This is the first report on mapping of CR genes in *B. nigra*. Two CR genes *Rcr6* and *Rcr8* were identified in *B. nigra*. Rcr6 was genetically mapped and SNP markers linked to the gene were developed.

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321
POSTERS THEME B

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Blackleg and clubroot – Disease resistance development in Cargill Specialty Seeds and Oils

Background: Blackleg (*Leptosphaeria maculans*) and clubroot (*Plasmodiophora brassicae*) are major diseases of canola across the western prairies of Canada. Current farming practices including tight rotations impose extreme selection pressure resulting in the shifting of pathogen populations. This has lead to more severe disease epidemics and the generation of new and increasingly more virulent pathotypes of these species, which puts the canola industry in western Canada at risk.

Objectives: Develop a plan to combine durable disease resistance breeding, strategic deployment of resistant canola hybrids along with integrated pest management to slow down the erosion of resistance against these two diseases and ensure the high yielding potential of Cargill canola hybrids.

Methods: New germplasm disease screening, evaluation of pathogen avirulence gene profiles, genetic marker-assisted breeding, indoor and outdoor evaluation of seedling and mature plant resistance under high disease pressure and long term resistance gene deployment were used to reach the goals of high yield and disease resistance.

Results: Blackleg disease resistance levels in both public disease trials at different geographic locations and through a series of post registration evaluations indicate that Cargill canola hybrids harbor high and durable resistance to blackleg. Meanwhile clubroot resistance in Cargill products is not only effective against the predominant pathotypes found in Alberta today, but also against new pathotypes recently identified.

Conclusions: Exploring different genetic pools and fully understanding the structure of pathogen populations greatly help in the deployment of effective resistance genes to control blackleg and clubroot. Combining major gene resistance with quantitative trait loci is the key for durable resistance.

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Effect of spore load on growth of clubroot-resistant canola and Napa cabbage

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Background: Clubroot caused by *Plasmodiophora brassicae* Woronin reduces yield in canola (*Brassica napus L.*) and *Brassica* vegetables such as Napa cabbage (*B. rapa L.* ssp. *pekinensis*). Genetic resistance is essential for clubroot management. However, studies indicate that high spore loads may reduce growth and delay development in clubroot-resistant cultivars of canola. (Deora et al. 2012, Hwang et al. 2011). This indicates that resistance to clubroot involves a high level of metabolic activity in the plant.

Objectives: To compare the growth of clubroot-resistant canola and Napa cabbage cultivars at two adjacent field sites that differ only in spore loads of *Plasmodiophora brassicae*.

Methods: One susceptible and three clubroot-resistant cultivars of canola and of Napa cabbage were direct seeded in soil naturally infested with *P. brassicae* at the Muck Crops Research Station, Holland Marsh, Ontario. Each trial was conducted as a randomized complete block design with four replicates. Plant growth was assessed weekly in canola by measuring plant height from hypocotyl (at soil surface) to shoot apex. Plant growth in Napa cabbage was assessed weekly by measuring the leaf length of the third and fourth youngest leaves. Area Under the Growth Stairs was calculated using weekly measurements. At 9 weeks after planting, the proportion of canola plants at selected developmental stages (vegetative, bud, flowering, pod development) was assessed. Plants were harvested and weighed and roots were assessed for clubroot incidence and severity using a standard 0 3 rating scale. The data were combined across trials and analyzed using a mixed model analysis of variance to examine the interaction between cultivar and location (PROC MIXED, SAS software version 9.1).

Results: There were no symptoms of clubroot in the resistant cultivars, but severe clubroot developed (100 DSI) in the susceptible canola control at both sites. At the location with a higher spore load (1 x 106 resting spores g-1 dry soil), the height of the resistant canola cultivars was reduced by 39% (_6 SE) and leaf length of Napa cabbage was reduced by 19% (_3 SE) relative to the site with a lower spore load (1 x 105 spores g-1 soil).

Conclusions: These field results support the observations from controlled environment trials (Deora et al. 2012) that high concentrations of resting spores of *P. brassicae* cause a reduction in the growth of clubroot-resistant cultivars of canola. A similar pattern of reduction was also observed for vegetative growth of Napa cabbage.

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323
POSTERS THEME B

Optimizing blackleg chemical control: Baseline sensitivity of QoI fungicides and effect of timing of application on disease control

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Background: Blackleg, a disease caused by the fungus *Leptosphaeria maculans* is resurging as an important threat to canola (*Brassica napus*) production in North Dakota. Most commercial cultivars planted in the region are resistant to *L. maculans* strains containing *Avrlm2* and/or *Avrlm3* but new strains capable of defeating these resistance genes are increasing in prevalence (Nepal et al., 2014). Consequently, the use of fungicides to manage this disease will increase in the near future as blackleg outbreaks intensify. Four of the five fungicides registered in North Dakota to control blackleg are Quinone-outside inhibitors (Friskop et al., 2015). Since many plant pathogens have developed resistance against fungicides of this group (Ma and Michailides, 2005), it is expected that *L. maculans* isolates with resistance to these compounds may develop in the future. To delay development of resistance to these compounds it is necessary to monitor changes in sensitivity and optimize fungicide applications.

Objectives: Develop baseline sensitivity information of *L. maculans* to azoxystrobin and pyraclostrobin and determine the association between timing of fungicide application on disease control.

Methods: Replicated trials to accomplish these objectives were conducted twice. Sensitivity of *L. maculans* isolates to these fungicides was estimated using the methodology described by Wise et al. (2008) and was expressed as the concentration that reduced spore germination by 50% (EC50). Mean and median EC50 values were calculated for samples collected between 2004 and 2012. To determine the relation between timing of application and efficacy of control, trials were conducted in greenhouse conditions. Cultivar Westar was inoculated with a mixture of five virulent isolates 12 days after planting and sprayed with commercial doses of the fungicides -2, 0, 2, 4, 8 or 16 days after inoculation. Disease severity was estimated 12 days after inoculation and prior to harvest.

Results: Sensitivity to azoxystrobin was normally distributed in the population sampled and had a mean EC50 of 0.07 µg ml-1 and a median of 0.08 µg ml-1. Estimation of sensitivity to pyraclostrobin is under way. Greenhouse trials indicated that both compounds provide the best protection when applied no more than four days after inoculation. Delaying fungicide application by 16 days increased plant mortality significantly.

Conclusions: North Dakota *L. maculans* populations are very sensitive to azoxystrobin at present time. Growers should apply fungicides as early as possible after the crop has emerged; delaying the application could compromise the efficacy of control.

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324
POSTERS THEME B

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Changes in pathogenic variability of *Leptosphaeria maculans* in North Dakota

Background: Blackleg, caused by *Leptosphaeria maculans*, is once again becoming a threat to the canola (*Brassica napus*) industry in North Dakota (del Rio Mendoza et al., 2012). This resurgence is powered by a shift in the virulence profile of *L. maculans* populations in the state that was first noticed in 2003 (Bradley et al., 2005). A more recent paper that documented the change by comparing the prevalence of strains from pathogenicity groups 2, 3, T, and 4 among isolates collected between 2004 and 2009 concluded the previously dominant PG-2 had been replaced almost completely by strains from groups 3 and 4 (Nepal et al., 2014). Strains from these groups are in general more aggressive and capable of overcoming resistance carried by most commercial varieties grown in the region (Nepal et al. 2014). The sequencing of *L. maculans* avirulence genes has made possible to determine their prevalence in populations using PCR assays (Gout et al., 2006; Fudal et al., 2007; Parlange et al., 2009; van de Wow et al., 2014). Combining traditional screenings with PCR assays could provide a more complete image of the variability present in *L. maculans* populations in North Dakota.

Objectives: Characterize the prevalence of pathogenicity groups and known avirulence genes in *L. maculans* isolates from North Dakota.

Methods: Single-spore cultures from *L. maculans* isolates collected in 2014 were evaluated in greenhouse to determine pathogenicity groups by inoculating them on differential cultivars 'Westar', 'Glacier', and 'Quinta' as described by Nepal et al. (2014). Screening these isolates with additional differentials is under way. Presence of avirulence genes *AvrLm1, AvrLm6, AvrLm4-7*, and *AvrLm11* is being carried out using primers and PCR assays described in the original papers.

Results: Preliminary results suggest that PG-4 continues to be the most predominant pathogenicity group in North Dakota. Assays to determine the prevalence of avirulence genes is under way.

Conclusions: Will be based on outcome of results.

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325
POSTERS THEME B

Control of Sclerotinia stem rot in oilseed rape: Initial investigations and plans for future work at the Centre for Crop and Disease Management

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Background: The fungus *Sclerotinia sclerotiorum* causes disease in over 400 species of plant including oilseed rape, in which it is commonly termed Sclerotinia stem rot (SSR). Disease only occurs when environmental conditions are suitable for fungal germination and growth, though *S. sclerotiorum* can remain soil borne for up to a decade in the form of tough resting bodies called sclerotia. In Australia, as oilseed production has increased in response to global demand, SSR incidence has been on the rise. There is now an urgent need to develop new ways of quelling yield losses attributed to this disease.

Objectives: With our fledgling research program at the Centre for Crop and Disease Management based at Curtin University, Perth, we plan to explore several lines of investigation regarding SSR management, with an aim of understanding key aspects of fungal pathogenesis. We will conduct an assessment of *S. sclerotiorum* global genetic diversity through a collaborative effort; this will include improvement of the *S. sclerotinia* reference genome with up-to-date sequencing technology. We will conduct a genome wide association study on *Brassica napus* varieties in an attempt to elucidate its genetic potential for SSR resistance. We will test fungicides that are not registered in Australia for their efficacy against locally collected fungal isolates. And we will use the model species *Arabidopsis thaliana* to identify the most promising genetic modifications for SSR resistance, with the overall aim of introducing these into *B. napus*.

Methods: Intergenic spacer (IGS) sequences of 70 *S. sclerotiorum* isolates collected from four regions in Western Australia were sequenced and compared. These isolates were then phenotypically screened using a mycelial compatibility group (MCG) test to determine whether those with similar IGS sequences were vegetatively compatible. Several fungicides currently unregistered in Australia for the control of SSR were tested in vitro on local *S. sclerotiorum* isolates and their EC50 concentrations were determined. The genome of the reference isolate was re-assembled using reads generated with Pacific Biosciences single molecule real time sequencing technology.

Results: The 70 *S. sclerotiorum* isolates fall into four distinct IGS groups, which have been identified in several other regions globally. These groups largely correspond to MCGs based on in vitro screening for vegetative compatibility. Unregistered fungicides of the strobilurin, azole and carboxamide classes have good activity against local *S. sclerotiorum* isolates in vitro. Re-sequencing of the reference genome allowed for closure of approximately 1 Mb of sequence gaps, leading to an improved and more reliable sequence.

Conclusions: IGS sequencing indicates that Australian *S. sclerotiorum* isolates share genetic similarities with the global population. Correlation of sequences with MCGs indicates no unusual propensity for outcrossing or other forms of genetic recombination in these isolates. There is rationale for field-trialling and registering several antifungal chemistries currently out of reach of Australian oilseed growers. The improved genome of *S. sclerotiorum* is close to the size predicted by optical mapping in a previous study. This new sequence will be an important resource for global isolate comparison and the *S. sclerotiorum* research community.

Genome sequencing provides insight into pathogenicity of *Plasmodiophora brassicae*

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Background: Clubroot disease of *Brassica* crops is caused by the biotrophic parasite *Plasmodiophora brassicae* (Pb). Infection of canola (*Brassica napus*) by Pb stimulates gall formation on the roots, which alters source/sink relationships, impedes water transport and results in significant yield loss in severely infected crops (Dixon, 2014). The most virulent pathotype of Pb on canola in Canada is pathotype 3 (Pb3), as defined on the differentials of Williams (1966). In contrast, pathotype 6 (Pb6) is virulent mainly on vegetable *Brassicas* (Strelkov and Hwang, 2014). As an obligate biotroph, Pb is highly adapted to supress host defence through secretion of effector proteins. Effectors are defined as small, secreted proteins produced by the pathogen to help with pathogen colonisation through the suppression of host immunity (Win 2012).

Objectives: Identification and functional characterization of Pb effectors

Methods: The genomes of Pb3 and Pb6 were sequenced from isolated resting spores using the 454-titanium platform. RNA from root tissues of *B. napus* cv. DH12075 infected with Pb3 was extracted at 1-week intervals (up to 6 weeks after inoculation), and the transcriptome sequenced by Illumina technology. A subset of predicted effectors was cloned and characterised by transient expression in planta.

Results: The Pb3 and Pb6 genomes were assembled into 109 and 356 scaffolds, respectively. The total number of predicted genes for Pb3 was 10,851 and for Pb6 10,070. A total of 590 effector genes were predicted for Pb3 with 317 of them ranging in size between 1000 bp to 300 bp. We have identified effectors that are able to suppress PAMP-triggered immunity (PTI) in a transient expression assay in tobacco leaves.

Conclusion: This study provides the first insights into the genome of *P. brassicae*, an obligate soil-borne parasite that has been difficult to study as it resides within the root tissue of its host. The Pb genome is small (24.2 Mbp) and highly compact with less than 2% repeats. We have identified a subset of genes as potential virulence factors and provided evidence for their function in the suppression of plant defence.

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327
POSTERS THEME B

Diversity of symptoms in *Arabidopsis* plants infected by a Canadian strain of AY-WB

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Background: Phytoplasmas are non-culturable bacteria that are able to infect many plant species including ornamentals, crops, and perennial plants. Plants that are infected by a phytoplasma species often develop symptoms ranging from yellowing, witches' broom, virescence, phyllody, stunting, little leaf, big bud or other symptoms. Insects (primarily leafhoppers) act as vectors to transmit the pathogen. Some of the symptoms caused by phytoplasma are strain specific while other symptoms are common features of phytoplasma infection among various host plants. The Aster Yellows phytoplasma strain Witches' Broom (AY-WB) infects many species of crucifer family including *Brassica napus* (canola/rapeseed) and *Arabidopsis thaliana*. AY infection can be devastating, leading to severe yield loss (Olivier et al., 2010), and it is of great concern that the frequency of AY infection in Canada is on the increase.

Objectives: Using *Arabidopsis* as a model to study AY-WB disease of canola.

Methods: The SK AY-WB strain of phytoplasma was transmitted to *Arabidopsis* Col-0 using the leafhopper species *Macrostella quadrillineatus* (Mq). Plants were subsequently observed for the development of the symptoms. The presence of the phytoplasma was detected in the infected plants as described by Dumonceaux et al., (2014). Moreover, the possibility of the transmission of phytoplasma through the seeds of the infected *Arabidopsis* plants was examined.

Results: *Arabidopsis* Col-0 plants showed a range of symptoms after infestation by Mq leafhoppers carrying SK AY-WB. The symptoms varied from stunting, anthocyanin accumulation, Yellowing, witches' broom, to phyllody, virescens, little leaf, big bud and apical dominance. The multiplicity of symptoms was surprising as it encompassed the symptoms of a wide diversity of phytoplasma strains on different hosts. Infected plants showed a drastic drop in the seed production, and the seeds were shrivelled in the heavily infected plants. Progeny of the plants with severe symptoms showed overall reduced growth, anthocyanin accumulation in younger leaves, and yellowing of the leaves compared to the healthy control plants. Traces of phytoplasma were monitored and detected by PCR in the progenies of infected plants.

Conclusion: This study provides the first evidence that SK AY-WB of Canola could infect *Arabidopsis* using infected *M. quadrilineatus* leafhoppers. Plants that were infected by the phytoplasma showed various symptoms typical of different phytoplasma species. The *Arabidopsis* – AY-WB pathosystem described here provides an interesting model to study the interaction of AY-WB with its host plant.

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Potential establishment of *Leptosphaeria maculans* (phoma stem canker) on Chinese oilseed rape

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Background: Phoma stem canker (blackleg), caused by *Leptosphaeria species*, is a serious disease of oilseed rape (*Brassica napus*) that produces considerable worldwide losses. In China, phoma stem canker has not generally been a serious problem and only the less damaging *L. biglobosa* has been isolated from diseased crops. However, it is possible that the more damaging *L. maculans* may spread to China since it has spread into areas where only *L. biglobosa* had been present, such as Canada or Poland (Fitt et al. 2008).

Results: Both *L. biglobosa* and *L. maculans* were detected on crop debris and seed in shipments of oilseed rape seed imported into China through Shanghai or Wuhan ports in 2009-2011 (Zhang et al. 2014). Incidence of phoma stem canker in pre-harvest surveys from 2005 to 2012 was greater on winter oilseed rape along the Yangtze River in central China (in May) than on spring oilseed rape in north China (in August). When the causal pathogen was isolated from stem cankers, it was always identified as *Leptosphaeria biglobosa* by morphology in culture and/or by species-specific polymerase chain reaction and no *L. maculans* was isolated. Descriptions of the observed spread of *L. maculans* into areas previously colonised by *L. biglobosa* across a spring oilseed rape growing region (Alberta, Canada, westwards, 1984-1998) were used to estimate the potential westward spread of *L. maculans* in China across spring oilseed rape growing regions (north China). Descriptions of the spread across a winter oilseed rape growing region (Poland, eastwards, 1984-2004) were used to estimate spread across a winter oilseed rape growing regions (central China). The rates of spread were estimated as 47 km per year across spring oilseed rape in north China and 70 km per year across winter oilseed rape in central China. Dispersal modelling suggested that the rate of spread of *L. maculans* across Alberta, Canada (c. 17 km per year) could be explained by wind-borne dispersal of ascospores.

Conclusion: It is important to develop strategies to decrease the risk of spread of *L. maculans* into China.

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329
POSTERS THEME B

Clubroot disease in rapeseed - A persistent challenge thanks to varying pathotypes

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Background: Clubroot, caused by *Plasmodiophora brassicae*, is a soil-borne disease of high importance for *Brassica* crops. Infestation with the pathogen causes thickening of roots and subsequent gall formation. On heavy infested fields a seed yield reduction of 50% or more can be observed. By today, the disease is wide-spread and prevalent on every continent. Clubroot infection can be monitored on ca. 8 % of the European cultivated rapeseed area. Clubroot became a major disease in North America, Australia, Asia and especially in Europe. High incidence of the disease is noted across Europe including regions in Poland, Germany, France, Czech Republic, UK, Denmark and The Netherlands.

Infestation with *Plasmodiophora brassicae* can be reduced by different means including wide crop rotation, high grade agronomical practice, weed control and amelioration liming. However, the method of choice to reduce damage on rapeseed caused by *Plasmodiophora brassicae* is breeding for resistant varieties (DIEDERICHSEN et al. 2009).

Pathotype-specific resistances in Europe: For success of resistant varieties, monitoring of pathotypes of *Plasmodiophora brassicae* in rapeseed cultivation areas is of prime importance. Pathotypes are quite similar phenotypically but differ towards their virulence against different host plants. Due to specific host-pathogene interaction it is possible to classify pathotypes. In Germany pathotype P1, P3 and P5 (according to SOMÉ et al. 1996) are the most common ones. European breeders used this knowledge for the development of new rapeseed varieties with resistance to the prevailing pathotypes. This work led to the development of two clubroot resistant rapeseed varieties, Mendel and Tosca registered in Europe by 2000. Due to the fact, that the resistance takes effect only on the prevailing pathotypes in Europe, it is called pathotype-specific resistance.

Challenges for resistance breeding in Europe: The introduction of Mendel to the European market helped farmers to cultivate rapeseed even on infested fields. Today's new clubroot-resistant varieties possess better seed yield and agronomic characteristics reflecting the investment of plant breeding into better control of the disease. Pathotypes of *Plasmodiophora brassicae* have the ability to widely adapt to environmental changes and to overcome the resistance of Mendel. Especially in Germany resistance breaks have been reported in different regions over the last years (ZAMANI-NOOR 2014). Today, it is the challenge for European rapeseed breeders to detect new sources of resistance against clubroot and to integrate them in new competitive rapeseed varieties.

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330
POSTERS THEME B

Clubroot control in oilseed rape using host resistance – potential and challenges

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Clubroot has gained significant relevance in European oilseed rape production and is expected to further increase its impact due to close crop rotations and climate change. To control this disease an integrated approach needs to be applied, which is primarily based on the use of resistant cultivars. A number of resistant cultivars has been released in the past decade, all of these are basically sharing the same resistance source. The performance of resistant cultivars in respect to agronomy and efficacy of their resistance towards local *Plasmodiophora brassicae* isolates will presented. Compatible races are present but so far these did not cause wide-spread break-down of resistance efficacy. Some future perspectives of clubroot resistance breeding will be addressed including new resistance sources from *Brassica* and Raphanus and clubroot reactions of interspecific/ intergeneric hybrids will be presented. Basic considerations on resistance management for this soil-borne disease and its application for clubroot resistance will be discussed.

331
POSTERS THEME B

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Leptosphaeria maculans effectors involved in the oilseed rape systemic colonization

Background: The blackleg, caused by the fungus *Leptosphaeria maculans*, is one of the most devastating diseases of oilseed rape. This disease is essentially managed through the breeding and use of resistant varieties. Specific resistance genes, interacting with the corresponding avirulence genes according to the gene-for-gene model, are used for genetic control in the fields. However the fungus may overcome these resistance genes. In that case, *L. maculans* colonizes the plant tissues, from the leaves to the crown, during a systemic and asymptomatic phase. It eventually switches to a necrotrophic stage leading to the damaging crown canker. The fungus may either successfully escape plant defenses leading to the stem canker development, or the plant can develop an "adult-stage" resistance (1) leading to a reduced stem necrosis incidence. To-date, we have no information on the fungal and plant genes involved in the molecular dialogue during systemic infection and stem canker development.

Objectives: Similarly to others models in which concerted waves of expression of effectors genes occur (2), we hypothesized that a set of fungal proteins, called "late effectors", are expressed only during systemic growth and/or stem necrosis development, and are necessary for the endophytic colonization of the plant. The objective of this project is to identify and characterize late effectors specifically expressed during the systemic colonization, and to understand whether "matching" resistance genes exist in the plant and may be involved in adult-stage resistance.

Methods: A RNAseq analysis evaluated fungal and plant genes expressed at three stages of the infection under controlled conditions (early infection, endophytic colonization and stem necrosis).

Results: *L. maculans* specifically expresses 165 genes encoding Small Secreted Proteins (SSPs) during stem infection. Except for their expression kinetics and genome location, these SSP display all characteristic features of early infection effectors like avirulence genes. Thus these SSPs are promising candidates as effectors involved in the systemic colonization. Among those 165 genes, 10 candidate effectors were chosen for further characterization based on their high level of expression, and their level of conservation in *L. maculans* populations.

Conclusions: These results suggest that successive waves of effector genes could be expressed by *L. maculans*, enabling it to carry out its complex life cycle. To elucidate whether late effectors could be recognized by plant resistance genes, it is envisaged to characterize and to genetically manipulate these late effector genes in order to express them during the early stages of infection. This could give us access to new tools for the identification of efficient resistance sources.

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Utilizing propidium monoazide (PMA) to differentiate viable and non-viable *Plasmodiophora brassicae* resting spores

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Background: Management of clubroot (*Plasmodiophora brassicae* Woronin) of canola (*Brassica napus L.*) on the Canadian prairies has proven to be difficult, partly due to the longevity of *P. brassicae* resting spores in soil. One way to quantify the number of soil-borne spores is by using quantitative polymerase chain reaction (qPCR) assays. However, qPCR does not distinguish between DNA from viable and non-viable spores. Propidium monoazide (PMA) has been used in conjunction with qPCR (PMA-PCR) to discriminate between viable and non-viable microorganisms (Moyne et al., 2013). PMA does not appear to readily cross through the external membrane of living cells, but seems to cross into non-viable cells of many microbes. Once inside the cell, photo-activation causes it to bind to double-stranded DNA. This alteration of the DNA inhibits subsequent amplification by qPCR.

Objectives: To assess the potential for using PMA-PCR to discriminate between viable and non-viable resting spores of *P. brassicae* for accurate quantification of viable spore loads.

Methods: Resting spores were isolated from clubbed roots of cabbage. Spores were subjected to heat treatments (80 °C for 0, 5, and 10 min) to produce a mixture of viable and non-viable spores. The spores were then treated with 0, 40, 80, and 120 µM PMA (Biotium Incorporated, Hayward, CA, USA) (Moyne et al., 2013). This was followed by qPCR analysis including a competitive internal positive control (Deora et al., 2015). The data were log transformed and tested for significance using ANOVA and Tukey's Honestly Significant Difference test at $P < 0.05$.

Results: Heat treatment did not affect estimates of spore concentration based on regular qPCR. However, estimates of resting spore concentration in samples heat-treated for 5 and 10 min were substantially reduced after PMA treatment. PMA-PCR with 40, 80, and 120 µM PMA estimated the spore number in samples that were not heat-treated at 2–5 x 106, whereas samples that were heat-treated for 5 min were estimated to contain 0.9–4 x 105 spores (62–96 % reduction), and samples that were heat-treated for 10 min were estimated to contain 0.2–1 x 105 spores (95–99 % reduction).

Conclusions: This study indicates that PMA-PCR may be useful in discriminating between viable and non-viable resting spores of *P. brassicae*. Further testing is required to determine if the results from PMA-PCR are consistent with bioassays of resting spore viability, and if this approach can be applied successfully to a wide range of soil samples.

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333
POSTERS THEME B

Vertical distribution of resting spores complicates clubroot management using fumigants

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Background: Clubroot (*Plasmodiophora brassicae* Woronin) has become an important disease in canola (*Brassica napus L.*) crops in western Canada. It is spreading quickly in central Alberta, but occurs in isolated patches in other regions.

Objectives: To examine the potential of fumigation to reduce / eliminate resting spores of *P. brassicae* in infested patches of fields and to assess the vertical distribution of resting spores in various soils.

Methods: Two replicated field trials were conducted in Ontario in 2014 to assess the efficacy of the fumigants metam sodium and chloropicrin in soils naturally infested with *P. brassicae*. Treatments of metam sodium and chloropicrin were applied at 15–20 cm depth. After application, the area treated with chloropicrin was covered with a totally impermeable film (TIF) for 14 days. The trials were then seeded with a susceptible host and rated using a 0-3 scale at 6 wk after planting.

In 2013, soil cores were collected to a depth of 53 cm from two sampling locations at each of three sites naturally infested with *P. brassicae*: two sites with mineral soil and one with high organic matter content. Three replicate cores were collected within 1 m2 at each sampling location. Resting spore concentration at selected depths in each core was initially assessed using standard qPCR methods. However, there were several instances of negative results from samples where spores were known to be present based on plant symptoms. As a result, a multiplex qPCR assay including a competitive internal positive control (CIPC) was developed to estimate the level of amplification inhibition in the qPCR reaction (Deora et al. 2015). This modification was used to assess each sample.

Results: Moderate rates of metam sodium provided excellent control of clubroot under controlled conditions (data not shown). Chloropicrin was only assessed in field trials because of applicator safety considerations. At a site where disease pressure was high, metam sodium had no measurable effect on clubroot severity, but chloropicrin substantially reduced severity and increased plant growth. At a second site where disease pressure was low, moderate rates of chloropicrin eliminated clubroot and increased plant growth. The variability among assessments of resting spore concentration in soil cores was high, both vertically and horizontally at all four sites.

Conclusions: Neither fumigant eliminated the pathogen at heavily infested sites. Reductions in clubroot severity will likely be more consistent where spore concentrations are lower and the treatment includes a TIF cover. Across all sites, spore numbers declined with increasing depth down to 20 cm depth, but were present to the bottom of each core (53 cm). Localized high spores concentrations, and spores deep in the soil profile, will make it difficult to use fumigants to disinfest sites, but also complicate activities for industries such as utilities and road construction that move large quantities of soil.

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334
POSTERS THEME B

Transcriptome and proteome analysis of *Leptosphaeria-Brassica* interaction

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Background: The hemibiotroph fungus *Leptosphaeria maculans* (blackleg) causes sever yield reduction on canola (*Brassica napus*). *L.maculans* penetrates the leave tissue through wounds and stomata. Hyphae grow in the intercellular space and move toward the stem as infection progress and eventually cause lesions at the base of the stem.

Objectives: Proteome and transcriptome of *L. maculans* and canola were carried out to understand the molecular aspects underlying the *L. maculans-B. napus* interaction.

Methods: Cotyledons of cultivar 'DH-Topas' that is susceptible to *L. maculans* isolate Lm00100 were sampled at 0, 2, 4, 6 and 8 days after inoculation (dai). RNA was extracted and sequenced using the Illumina HiSeq 2500 platform. RNA-sequence reads were mapped to the host and pathogen genome using CLC Genomics Workbench (1). The statistical software, DESeq2 (2) was used to determine the differentially expressed genes. Protein extracts of apoplastic fluid of cotyledons were subjected to label-free quantitative proteomics analysis to determine the protein profile of *L. maculans* during the same time course.

Results: We catalogued the expression profiles of 101040 *B. napus* and 12469 *L. maculans* genes. Among the differentially expressed genes were hydrolytic enzymes, nutrient transporters and a large number of *L. maculans* small-secreted protein-encoding genes.

Conclusions: We applied RNA-sequencing in combination with label-free quantitative proteomics and were able to gain information to better understand the pathogenicity of *L. maculans*. Patterns of gene expression during infection provided insight into the colonization and acquisition of nutrient by the pathogen and regulation of pathogen effector genes.

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335
POSTERS THEME B

Pyrethroid resistance of pest insects in oilseed rape in Germany

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Pyrethroid resistant pollen beetles (*Meligethes aeneus*) are widely distributed in Germany after the first resistant beetles were detected about 14 years before. 53 % of populations sensitive in biotests in 2005 declined to 0 % in 2011 already. Very clear resistance increased quite fast from 33 % in 2005 to 99 % in 2014. One reason of the fast development and spread of resistance are limitations in the use of other modes of action in oilseed rape, high infestation pressure in some years and regions, and too many sprays ignoring damage thresholds. In addition only pyrethroids are able to provide sufficient control for some of the pests occurring during the growing season and some products cannot be applied during the flowering period because of bee issues. Results of a monitoring program on pollen beetles show a still increasing resistance of the beetles. Monitoring of the other pest insects of oilseed rape in Germany showed no resistance yet in pest species including *Ceutorhynchus napi* and *C. pallidactylus, Dasineura spp*. and *Phyllotreta spp.*, but a clear resistance to pyrethroids has developed in *C. obstrictus* and *Psylliodes chrysocephala* since some years. Resistance of both species has not spread over the whole of Germany yet and resistance factors in laboratory biotests are far below resistance values known for *Meligethes aeneus.*

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Arabidopsis species provide insights for anticipatory breeding of durable white rust resistance in *Brassica crops*

Background: Major effect genes that encode receptor-like proteins can potentially provide long-lasting resistance to a particular pathogen, if they can detect specific molecules that are vital (highly conserved or slowly evolving) to pathogen fitness. WRR4 in *Arabidopsis thaliana* provides a *Brassicacea* example. An allele of this TIR-NBS-LRR gene from accession Columbia confers White Rust Resistance to four specialised races of *Albugo candida* from different *Brassicacea* hosts (Borhan 2008). An *Albugo* protein was predicted which is encoded by a conserved allele in all four races and is detected by the WRR4-Col protein. WRR4-Col is functional in transgenic oilseed *brassicas* (Borhan 2010).

Objectives: Every pathogen is capable of evolving new virulence, so combined effect from complementary R-genes is essential for truly durable disease resistance. Early identification of virulent pathogens that can overcome individual R-genes is therefore essential to anticipate which alleles could be assembled for the most effective R-gene combination. Our objectives have been to identify WRR4-virulent isolates of *Al. candida* from wild host species, and to use these to identify the next complementary R-gene.

Methods: White rust in *A. thaliana* is usually caused by *Al. laibachii* under field conditions. However, we attempted to collect *Al. candida* by springtime sampling of white rust from floral tissue in natural populations of *A. thaliana* that were growing in close proximity to Capsella bursa-pastoris (a prolific source of *Al. candida*). We also used perennial *Arabidopsis* species as a pot-grown bait to trap white rust throughout the year. PCR-based markers were used to identify *Al. candida* from diseased tissue. When identified, an isolate was propagated in *Arabidopsis* accessions lacking WRR4-resistance, and then tested for virulence in Columbia. If found, then Col-0 virulent isolates could be used in the next round of R-gene identification by screening a global collection and recombinant inbreds of *A. thaliana* for association and recombination mapping of resistance.

Results: Floral tissue proved to be a useful source for *Al. candida* from natural infections of *Arabidopsis.* Although isolates were difficult to propagate and Col-virulence was rare, three have been identified including one from a natural population of *A. thaliana* and one each from bait plants of *A. lyrata* and *A. halleri.* Resistance to all three isolates has been mapped to the WRR4 locus which contains two additional TIR-NBS-LRR genes.

Conclusions: An alternative allele of WRR4 or an allele from a neighboring gene may provide broader spectrum resistance to *Al. candida*. Combining this allele with the Col-allele could provide a durable combination for white rust resistance in transgenic brassica crops. The new isolates are avirulent in brassicas and are therefore not a direct threat to resistance that already occurs crops. However, identifying the causal mutation for Col-0 virulence would be useful for diagnostics to detect recombinants in pathogen populations that could potentially threaten crop production. Genome analysis of Col-virulent isolates will be used to determine whether causal mutations occur in a conserved effector protein of *Al. candida*.

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337
POSTERS THEME B

Effects of quantitative resistance on R gene-mediated resistance against *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape)

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Background: Phoma stem canker, caused by *Leptosphaeria maculans*, is a damaging disease on oilseed rape in the UK and can cause up to 50% yield losses (Fitt et al., 2011). Use of durable host resistance to control this disease is becoming more important. Resistance against *L. maculans* may be major resistance (R) gene-mediated resistance or quantitative resistance (QR). R gene-mediated resistance is race-specific and often rendered ineffective due to pathogen population changes from avirulent to virulent (Sprague et al. 2006). QR is race non-specific and is considered more durable but it is a partial resistance. Recent work showed that QR decreased the speed of increase in frequency of virulent alleles overcoming Rlm6 resistance against *L. maculans* (Delourme et al. 2014). This suggests that QR affects the effectiveness of R gene resistance.

Objectives: To investigate effects of QR on resistance against *L. maculans* mediated by different R genes.

Methods: Eight winter oilseed rape cultivars with different types of resistance were used in field experiments at 11 different sites in the 2010/2011, 2011/2012 and 2012/2013 growing seasons. Six cultivars had an R gene in a background with or without QR: DK Cabernet (*Rlm1* + QR), Capitol (*Rlm1*), Adriana (*Rlm4* + QR), Bilbao (*Rlm4*), Excel (*Rlm7*+ QR) and Roxet (*Rlm7*). Two cultivars had QR without known R genes: Es-Astrid and NK Grandia. The field experiments were arranged in randomised block designs with three replicates. The severities of phoma leaf spots in autumn and stem canker in summer were assessed.

Results: For cultivars carrying *Rlm1* or *Rlm4*, which were only partially effective because the frequencies of the corresponding avirulent alleles of *AvrLm1* or *AvrLm4* in *L. maculans* populations were less than 50%, there was no effect of quantitative resistance on severity of phoma leaf spots but there was an effect on severity of stem canker. The severity of stem canker on DK Cabernet (*Rlm1* + QR) was less than that on Capitol (*Rlm1*), suggesting that *Rlm1* is more effective when it is introduced into a host background with QR than without QR. Similarly, less severe stem canker on Adriana (*Rlm4* + QR) than on Bilbao (*Rlm4*) suggested that *Rlm4* is more effective when it is introduced into a host background with QR than without QR. Cultivars Roxet and Excel both carried an effective resistance gene *Rlm7*, because the frequency of the corresponding avirulent allele of *AvrLm7* in *L. maculans* populations was greater than 97%, there were no significant differences between them in severities of both phoma leaf spots and stem canker. Of the two cultivars with only QR, Es-Astrid developed less stem canker at most experimental sites than NK Grandia.

Conclusions: There were effects of background quantitative resistance on the effectiveness of R gene-mediated resistance in *Brassica napus*. Quantitative resistance increased effectiveness of R gene-mediated resistance against *Leptosphaeria maculans* in oilseed rape.

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Effects of temperature on R gene-mediated resistance against *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape)

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Background: Phoma stem canker (*Leptosphaeria maculans*) is an economically important disease on oilseed rape (*Brassica napus*) in Europe, Australia and North America. Effective control of this disease relies on use of two types of resistance; major resistance (R) gene-mediated qualitative resistance and quantitative resistance (QR) (Delourme et al. 2006). However, effectiveness of host resistance is known to be affected by environmental factors, including temperature. *Rlm6*-mediated resistance to *L. maculans* is effective at 20°C but not at 25°C (Huang et al. 2006). At least 16 R genes (*Rlm1-Rlm11, Rlms, LepR1-LepR4*) have been identified in *Brassica* species but only two (*LepR3* and *Rlm2*) have been cloned (Larkan et al. 2013, 2015). With predicted global warming, there is a need to investigate effects of temperature on effectiveness of R gene-mediated resistance against *L. maculans*.

Objectives: To investigate effects of temperature on effectiveness of resistance against *L. maculans* mediated by different R genes.

Methods: Oilseed rape cultivars or breeding lines with different R genes in backgrounds with or without QR were inoculated at 20°C and 25°C. Cotyledons of 12-day old plants were wounded and a 10 µl drop of 107 spores/ml conidial suspension placed over the wound. Symptoms were assessed at 16-18 days-post-inoculation on a 0-9 scale (0: no symptoms; 9: large grey lesions with pycnidia). To avoid the effects of background QR and investigate plant defence responses, near-isogenic lines of Topas (susceptible) with R genes *Rlm4* (Topas-*Rlm4*) or *LepR3* (Topas-LepR3) were used. Cotyledons of 12-day-old plants were each infiltrated with 10 µl of 106 spore/ml conidial suspension at 20°C and at 25°C.

Results: There were differences in temperature-sensitivity between the ten different R genes *(Rlm1, Rlm2, Rlm3, Rlm4, Rlm5, Rlm6, Rlm7, LepR1, LepR2* and *LepR3*) tested and there were differences in response to temperature for the same R gene in cultivars with or without quantitative resistance. There were differences in defence responses between Topas-*Rlm4* and Topas-*LepR3*, with Rlm4 responding more quickly and more strongly than *LepR3* at the higher temperature.

Conclusions: Background QR affected the temperature-sensitivity of R gene-mediated resistance. Understanding effects of temperature on interactions between hosts and pathogens will help breeding of cultivars with durable, temperature-resilient resistance.

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339
POSTERS THEME B

Fine mapping of a clubroot resistance gene using SNP markers based on RNA-seq

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Background: Clubroot disease, caused by *Plasmodiophora brassicae*, is one of the most devastating diseases of canola (*Brassica napus, B. rapa*) in western Canada. It is difficult to control using chemical treatments because the pathogen can persist in the soil as resting spores for many years (Voorrips 1995). Therefore the development of resistant cultivars is considered to be the most effective way to control the disease. However, sources of clubroot resistance (CR) in canola are very limited. Interestingly, materials effectively resistant to a broad range of pathotypes of *P. brassicae* have been identified in one of the *B. napus* progenitor species, *B. rapa* (Peng et al. 2014). Objectives: A Chinese cabbage cultivar "Jazz" showed strong resistance to five pathotypes of *P. brassicae* prevalent in Canada (Peng et al, 2014). In this study, we identified a CR gene namely *Rcr2* in "Jazz" and genetically mapped the gene using bulked segregant RNA-Seq (BSR-Seq) analysis. SNP markers tightly linked to the CR gene were developed for use in marker assisted selection (MAS).

Methods: A single resistant (R) plant was crossed with a doubled haploid *B. rapa* canola susceptible (S) line ACDC and the resulting F1 was back-crossed with ACDC to produce a BC1 population. Pathotype 3 of *P. brassicae* was used for inoculation. Leaf tissue from 30 R and 30 S plants were collected and bulked respectively as a biological replicate, with three replicates. RNA was isolated from each bulk using RNeasy Plant Mini Kit (Qiagen). BSR-Seq was performed following the methods described by Liu et al (2012). Validation and genotyping of SNPs were carried out using Kompetitive Allele Specific PCR (KASP) method (http://www.lgcgroup.com/). Genetic distance was determined with JoinMap 4.1.

Results: A total of 9.76 Gb and 10.76Gb raw reads from R bulks and S bulks were obtained respectively. Approximately173 K SNPs were identified between R and S bulks. One significant peak was observed on 23-26 Mb of chromosome A03, which was predicted to contain the causal gene *Rcr2* in the region via BSR-Seq. A total of 302 informative genes with 1269 SNPs were detected in the region. Subsequently the BC1 population consisting of 1000 plants was genotyped with 17 SNPs using KASP method and *Rcr2* was fine mapped between two SNP markers SNP_A03_19 and SNP_A03_67 , 0.1 and 0.3 cM from *Rcr2*, respectively. The physical distance between the two SNPs is 0.21Mb.

Conclusions: A large number of SNPs tightly linked to *Rcr2* were identified through RNA-seq. Fine mapping of *Rcr2* in the study will enable cloning of the gene.

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Plasmodiophora brassicae in soil, water and OSR plants from Poland – bioassay, LAMP and qPCR detection

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Background: Clubroot has become one of the most serious threats to OSR cultivation in Poland. The disease is caused by a damaging protist, *Plasmodiophora brassicae* Woronin.

Objectives: The aim of this work was to survey agricultural soils and water from natural reservoirs close to agricultural fields as well as plants in arable fields to determine the occurrence, quantify the biomass and identify the races of *P. brassicae.*

Methods: Plant samples were collected in 2010-2014 from 256 fields of oilseed rape. Agricultural soils were collected randomly from 1168 fields. Water samples were collected from 9 reservoirs, including 6 ponds and 3 drainage ditches, as well as from 6 small puddles in OSR fields. Infestation of all soil samples was studied using a biotest. Out of these 59 samples of soils collected in Poland and one soil sample from Sweden, of known high level of infestation by *P. brassicae*, were also assessed using approaches based on Loop-mediated Isothermal DNA Amplification (LAMP) (Kaczmarek et al. 2014) and a quantitative real-time PCR method with Taqman probes (Wallenhammar et al. 2012). Races of the pathogen were evaluated according to Somé at al. (1996).

Results: Bioassays, LAMP and qPCR techniques differed with sensitivity, but all detected successfully the pathogen. Additionally bioassays facilitated studies on its races. Plants of OSR with advanced symptoms of clubroot were numerous on farmers' fields and were also detected on roots of varieties regarded as resistant or tolerant to *P. brassicae*. The concentrations of the pathogen DNA in some soils reached 459 pg g-1 of dry soil, which exceeded 280 million of spores in soil volume. High amounts of *P. brassicae* DNA were also detected in water from puddles collected on fields with infested soil. In water reservoirs *Aspergillus fumigatus, A. viridans, A. niger* and *Rhizopus nigricans* were also present. Clubroot disease occurred in all the major OSR cultivation areas of Poland, including regions previously regarded as safe or free from the disease. Fields with numerous plants with clubroot symptoms were found in Pomerania, Opole region, Lower and Upper Silesia, Varmia and Mazury. While pathogen incidence was low in other regions, but no region of Poland was exempt from the disease. Apart from OSR, clubroot was also observed on field-planted Chinese cabbage and broccoli. On the whole, the pathogen was found in 86 out of 295 counties (29.2%) that were monitored using a biotest. Extensive screening for races done in 2014 found the prevalence of P1 and P3 (88%) in comparable amounts in most regions, even though races P2, P4 and P5 were also detected in some locations. Regardless of pathogen race, all the methods used detected successfully *P. brassicae* in tested materials.

Conclusions: *Plasmodiophora brassicae* is widespread in Poland at very high levels of host and environment infestation. There is a high demand for OSR cultivars with multiple resistance to several races of the pathogen.

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341
POSTERS THEME B

Exploiting mycoviruses to control Stem rot of oilseed rape caused by *Sclerotinia sclerotiorum*

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Background: Stem rot caused by *Sclerotinia sclerotiorum* is the most important disease on oilseed rape in China. Due to no resistant-cultivar is available, the loss caused by stem rot every year in China is very huge, about 2 billion $ RMB (or 0.3 billion US$) per year. It is necessary to seek for efficient way to control stem rot. Mycoviruses are viruses that infect fungi and are common in nature. Hypovirulence-associated mycoviruses when released in field may cause debilitation of fungal pathogen, thus, they have a potential to control fungal crop diseases (Xie and Jiang, 2014). However, mycoviruses are transmitted vertically via host reproduction or horizontally via host hyphal anastomosis which only occurs between vegetative compatible individuals. Thus, the efficacy of mycoviruses in field is limited by host vegetative incompatibility reaction. Since the vegetative compatibility of *S. sclerotiorum* in field is very complicated, it may not very easy to exploit hypovirulence-associated mycoviruses to control stem rot of oilseed rape.

Objectives: The objectives are screen hypovirulence-associated mycoviruses which could transmit and spread in field efficiently and exploit them to control stem rot of oilseed rape.

Methods: Strains of *S. sclerotiorum* were originally isolated from sclerotia produced on the lesion of diseased plant. Strains showed abnormal phenotype were screened to detect if they were infected by mycoviruses either with RNA_Seq analysis, or extraction of viral genome RNA and DNA from hyphae. Mycoviruses horizontal transmission test were conducted with dual culture on PDA plate. Mycoviruses that could be efficiently transmitted among host's different vegetative compatibility groups were further tested their potential to control stem rot of oilseed rape by spraying hyphal fragments suspension on plants of oilseed rape, and then inoculating virulent strain on the same plants.

Results: More than 160 mycoviruses were detected in *S. sclerotiorum*, these mycoviruses either have dsRNA genome, or (+)ssRNA genome, or (-)ssRNA genome or ssDNA genome. Some mycoviruses could be grouped into existing Families, while some mycoviruses were novel, and their classification status were uncertain. Among them, two mycoviruses showed strong infectivity. One is unclassified geminivirus-like DNA virus, *Sclerotinia sclerotiorum* hypovirulence associated DNA virus 1(SsHADV-1) (Yu et al, 2010); another is a typical partitivirus, *Sclerotiorum sclerotiorum* partivirus 1 (SsPV1) (Xiao et al, 2014). Field tests showed that SsHADV-1 could control stem rot efficiently (Yu et al, 2013); the field test for SsPV1 will be conducted in March this year.

Conclusions: Various mycoviruses could infect *S. sclerotiorum*, and some of them could be used to control stem rot of oilseed rape. Our study also suggested that mycoviruses may be an important force to suppress the virulence and population of *S. sclerotiorum* in nature.

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RNAi-induced resistance against insect pests in rapeseed

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Background: As one of the major crops worldwide, rapeseed/oilseed rape/canola is exposed to various biotic and abiotic stress factors, causing heavy yield losses. Damage by insects represents a serious threat for farmers. The pollen beetle (*Meligethes aeneus*), one of the most harmful insect pests in Europe, can cause yield losses of up to 50%. The most frequently applied plant protection strategy constitutes the use of various classes of insecticides, particularly pyrethroids. Heavy pesticide usage causes not only negative impacts on the environment, but may also facilitate fast development of insect resistance against specific chemical classes. New strategies are therefore needed to control insect pests in rapeseed.

Objectives: Different technologies based on genetic engineering can potentially help to improve plant resistances to insect pests in order to support classical breeding methods. RNA interference (RNAi) using double-stranded (ds) RNA has been demonstrated as a promising method for pest control in crops.

In this study we aim for proof of concept that RNAi can induce highly-specific resistance against the pollen beetle whilst protecting beneficial organisms.

Methods: Candidate genes essential for beetle fitness were identified from gut-expressed pollen beetle expressed sequence tags, based on homology to genes showing lethal knockouts in the model beetle Tribolium (*T. castaneum*). Potential candidates were tested and analyzed by *in vivo* feeding trials with adult pollen beetles collected from flowering fields. Daily mortality rate was measured for 14 days after oral application of various concentrations and different dsRNA molecules derived from candidate target sequences.

After validation of the best candidate sequences, an RNAi-construct was created containing the target sequence coupled with inverted pollen-specific promoter sequences. The plasmid was used to generate insect-resistant prototype plants via Agrobacterium-mediated transformation.

Results: Candidate genes and pollen-specific promoter sequences were successfully identified.

Potential lethal-acting target genes of the pollen beetle were identified, isolated and tested for lethal effects, specificity and mode of action.

Feeding applications confirmed the proof of concept by demonstrating a significant reduction of beetle vitality after oral uptake of dsRNA from target genes.

The best potential target gene sequences have been transferred and integrated into the genome of *B. napus*, opening the way for first tests with prototype plants expressing promising RNAi targets.

Conclusion: Alternative pest control strategies are increasingly necessary to reduce pesticide applications, for protection of the environment and non-target organisms, to avoid insecticide resistances and therefore, to decrease yield losses caused by major insect pests. A suitable approach to breeding for pollen beetle resistance could be to breed plants expressing beetle-specific dsRNA sequences in the pollen. After feeding on pollen, activation of the RNAi mechanism can lead not only to immediate lethal effects, but also to long-term reduction in the fitness of beetle populations.

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343
POSTERS THEME B

Identification of genome-wide pathways for enhancing clubroot (*Plasmodiophora brassicae* Woronin) resistance in *Brassica napus*

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Background and Objectives: *Plasmodiophora brassicae* Woronin is an obligate pathogen that causes clubroot disease in *Brassica napus L.* and other *Brassica* crops. This disease is a threat for canola production in Western Canada. Buidling durable genetic resistance to this disease is an objective of many research groups. We present our multidisciplinary studies on the genetic and molecular aspects of the disease: root transcriptomics; microRNA analysis and biochemical analyses of two parental lines of *B. napus* differing in susceptibility to *P. brassicae* pathotype 3 and in a biparental population of the two lines.

Methods: Three disease developmental stages were investigated: primary (10 Days after Inoculation, DAI), secondary (22 DAI) and advanced disease development stage (42 DAI). Transcriptomics identified differences between the parents and pools of susceptible and resistant doubled-haploid segregants of a mapping population.

Results: Resistance response at the molecular level was evident even at the primary stage, the secondary and advanced disease development stages showed greater level of expression of PR proteins, jasomonic acid (JA), salicylic acid (SA) and signaling. In conjunction with phytochemical analyses, cell wall lignification was identified as a significant response in clubroot resistance. Disease susceptibility in contrast, was associated with higher expression of sugar breakdown and hexose transport, auxin metabolism and transport indicating pathogen modulated transcriptome level changes for subverting host nutrition. The susceptible parent and the DH pools of susceptible lines showed substantially greater number of differentially expressed genes at the secondary infection stage.

Conclusions: The lesser perturbance to gene expression in the resistant lines suggest stronger influence of the resistance gene derived from cv. Mendel in the spring *B. napus* canola. MicroRNA analysis at secondary infection stage indicated genome-wide differences for their targets in resistant and susceptible reaction. A model that incorporates the inferences is presented.

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Recurrent selection for resistance against mustard aphid, *Lipaphis erysimi* (Kaltenbach) in a set of *Brassica juncea - B. fruticulosa* introgression lines

Background: Mustard aphid, *Lipaphis erysimi* (Kaltenbach) is a key pest of oilseed *Brassica* in India. At present there is no genetic solution to the management of this pest. The development of aphid resistant cultivars is considered critical for effective and environment friendly method of pest management. A wild crucifer, B*rassica fruticulosa* was previously found to be resistant to mustard aphid (Kumar et al. 2011). A complete set of introgression lines was developed using single pod descent method following first cycle of backcrossing. The objective of this breeding scheme was to obtain optimal coverage of *B. fruticulosa* genome in the background of *B. juncea* with high level of aphid resistance.

Objectives: To enhance the level and stability of introgressed resistance against mustard aphid.

Methods: A set of 533 introgression lines developed from selfing of the BC_1 progenies of *B. juncea* / (*B. fruticulosa*/ *B. rapa*) combination were screened under field conditions during 2009-10 crop season. The lines showing resistant reaction were further screened in the successive crop seasons from 2010-11 to 2013-14 to identify introgression lines with consistent reaction. Aided epiphytotic conditions were created by artificial release of aphids @ 20 aphids/ plant. Five plants per replication were selected at random to study aphid population, injury symptoms on 0-5 scale and per cent plant infestation following the procedure of Bakhetia and Sandhu (1973).

Results: Proportion of plants harbouring lower aphid population was higher during 2009-10 but there was a general decline in the proportion of such plants in each successive year. While in the first three years, the maximum aphid population varied from 175.5 to 330.0 aphids/ plant, in the 2013-14 crop season, only 55.1 aphids/ plant were observed on selected resistant lines. Almost similar trend was observed w.r.t. Aphid Infestation Index (AII). However, the results from the parameter of per cent plants infested were not categorical. Out of the initial 533 introgression lines, 8 lines showed consistent resistant reaction over five cropping seasons. These included: Ad3L61, AD3L370, Ad3L401, Ad3L460, AD3L462, AD3L503, AD3L506, Ad3K190. These inbred lines had the lowest score for aphid population/ plant, per cent plant infestation, AII and fell in the resistant category. QTLs associated with aphid infestation index have been identified and the data will be presented.

Conclusion: Recurrent selection of introgression lines with lower aphid infestation over five years resulted in significant improvement in the level of introgressed resistance against mustard aphid.

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345
POSTERS THEME B

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Potential of *Brassica carinata* as a trap crop for managing large white butterfly, *Pieris brassicae (L.) on Brassica juncea*

Background: The large white butterfly, *Pieris brassicae (L.)* is an important pest of oilseed *Brassica* after mustard aphid in this part of country (Kumar 2011). "Alternative" environment friendly pest management methods to manage insect pest populations and to improve habitat diversification have received considerable attention in recent years. Several of these strategies exploit the differential host preference by many insects. In this strategy, trap crop is planted near to main crop which results in the reduction in the number of insect-pests reaching main crop by concentrating them on the trap crop. This approach enables a reduction in the amount of insecticide required for pest management or may negate the need for insecticides altogether. Previous observations at this institution have shown that *P. brassicae* exhibits differential oviposition preference on *B. carinata* over *B. juncea*.

Objectives: To test whether *B. carinata* can be used as an effective trap crop against *P. brassicae*.

Methods: *Brassica juncea* cv. PBR 210 was grown at Punjab Agricultural University, Ludhiana, India during 2012-13 and 2013-14 crop seasons. There were two treatments: *B. juncea* bordered by four rows of *B. carinata* cv. PC 5 and the other left unbordered. There were three replications for each treatment in a plot size of 6 x 5 m planted in a randomized complete block design. At the pest appearance, data on the number of *P. brassicae* larvae per plant were recorded at weekly intervals from ten plants per replication selected at random. Yield data were recorded at harvest.

Results: *B. juncea* plots bordered with *B. carinata* harboured significantly less number of larvae than unbordered *B. juncea* plots. In 2012-13 crop seasons, the mean larval density on *B. juncea* bordered with *B. carinata* was 1.5 larvae/ plant as against significantly high larval density of 10.7 larvae/ plant on unbordered *B. juncea* plots. Interestingly, the larval density on *B. carinata* border rows was exceptionally high (50.4 larvae/ plants) indicating the increased preference of butterflies to oviposit on *B. carinata*. The grain yield from bordered plots (1782.5 kg/ha) was also significantly higher than that from unbordered plots (1322.8 kg/ha). Almost similar trend was observed in 2013-14 crop season both for larval density and grain yield. Laboratory experiments have also indicated increased preference of butterflies for oviposition on *B. carinata*.

Conclusion: Since *P. brassicae* shows increased oviposition preference for *B. carinata*, this *B. carinata* has potential to be used as a trap crop to attract this pest. However, there is a need to establish the spatial and temporal pattern of deployment which will ensure the most effective trap crop system.

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Design and implementation of web-based open access rapeseed-mustard bibliographic information system

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Background: The advancement of research in the field of rapeseed-mustard diseases is clear to everyone. The numbers of articles are being published each year, resulting in increase of knowledge at every moment. Problem identification, future planning, implementation, and interpretation of individual research studies all depend on ready access to all of the relevant existing rapeseed-mustard diseases research knowledge. Need, felt to collate vast and scattered information published in several publications on different aspect of research on rapeseed-mustard disease in the form of bibliography database. An online bibliographic database (an organized digital collection of references to published literature in specific research domain) may be general in scope or cover a specific research discipline and usually focus on a particular domain of knowledge, and contain various types of publications including journal, conference proceedings, reports, newspaper articles, patents, books, government and legal publications, etc. [Ng et al., 2010].

Objective: The broad objective to develop this online bibliography database is to enabling researchers in the field of rapeseed-mustard disease to gain access to research articles data in this field, the long-term mission is to acquire existing and new research result and convey for new studies to the scientific community, students, and research centers in particular and our whole society in general.

Methods: The design of a bibliographic information system has to take into account two possible requirements: the completeness of the data held in the database, and ease of use for the intended users. System design and development usually proceeds through several phases of a software development life cycle (SDLC) that includes: feasibility study (problem identification); requirements analysis (users' requirements); choosing the system design and architecture; testing; implementation and evaluation . The system design is based on 3-tier architecture, separating data, logic and presentation tiers. This makes the application easier to maintain in the future, as well as to further upgrade with new features. HTML,CSS, MySql and PHP was used in implementing this bibliographic information system. (Oguntoyinbo, et al, 2013)

Results: The system developed RMBiblio, offer researchers easy and fast access of most comprehensive resource for research articles in rapeseed-mustard disease research. System supports a wide range of publication types, and has features like advanced search option, extraction of publications statistics based on a variety of visual form based queries, etc. Metadata formats suitable for describing scientific publications have been used in creating the database. Searching facility has been implemented using MySql full text search options, the rows returned are automatically sorted with the highest relevance first. HTML, CSS, was used for development of user interface; PHP is the middleware and MySql for the backend.

Conclusions: RMbiblio provides much needed exposure to rapeseed-mustard disease research published and acts an important resource to rapeseed-mustard information seekers. The system is receiving response from the rapeseed-mustard community both at national as well as international level.

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347
POSTERS THEME B

Effect of blackleg resistance on dispersal of *Leptosphaeria maculans* at the landscape scale

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Background: Previous studies have indicated that introduction of *Leptosphaeria maculans* to China may result in widespread dispersal there. Data collected from over 700 rape crops in Alberta, Canada from 1985 to 1998 have been used to simulate the potential for dispersal through China (Fitt et al. 2008), but these models have not accounted for effects of host resistance.

Objectives: Measures taken in Alberta in the early 1980s to slow spread of blackleg through the province may be of value to China. One of the core strategies of the Alberta plan was to encourage the development and deployment of blackleg resistant cultivars. Here, we focus on the potential benefits to China of genetic resistance and crop rotation on dispersal of blackleg disease.

Methods: We modelled *L. maculans* spread westwards from a single entry point across a 500km × 250km grid. A potential of 200-400 spores per unit percentage increase were spread throughout the simulated landscape with random angle. The distance travelled by each ascospore was determined by the inverse of the half Cauchy distribution (Savage et al 2012, Lo-Pelzer et al 2010, Xu and Rideout 1998), of which the parameter mu was used to fit the model to the empirical rate of spread, as determined from the Alberta data. Spores landing on a plant in a particular block were assumed to cause infection if a generated random number was less than InfRate, the parameter we used to adjust host resistance. Final disease prevalence and average distance of spread were calculated over each series of 100 simulations.

Results: At an InfRate of 0.25, 82% of the simulations showed virtually no spread over 15 years, i.e. spread of blackleg through a landscape dominated by highly resistant cultivars is expected to be essentially zero. The percentage of "no-spread" simulations decreased to 20%, 8%, and 5% when Infate parameter values were increased (i.e. cultivar resistance decreased) to 0.50, 0.75, and 0.99, respectively.

Conclusions: Employment of resistant cultivars appears likely to effectively reduce the rate of blackleg dispersal in the event of an introduction of *L. maculans* to China.

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348
POSTERS THEME B

Modulation of the resistance QTL effects under abiotic stresses: Case study of resistance to clubroot in rapeseed under nitrogen constraint.

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Background: The use of resistant varieties is a major component of integrated strategies for the control of plant diseases. In a perspective of low input agriculture development, taking into account abiotic challenges in the evaluation of resistant factors is essential for constructing and managing resistant varieties in different cropping systems.

Objectives: The aim of this work was to estimate the impact of nitrogen constraint on the genetic architecture of clubroot resistance in *Brassica napus*.

Methods: 108 doubled-haploid (DH)-lines derived from the cross between Darmor-bzh (harbouring partial quantitative resistance to clubroot caused by *Plasmodiophora brassicae*) and Yudal (susceptible) were tested for clubroot resistance under two N conditions (N+= 8 mM of nitrate vs N-=1 mM) and using two single spore isolates (K92-16 and eH). The overall experiment was carried out twice each with three replicates. Disease scoring was done 49 days post inoculation. For each isolate and each year a mixed linear model was fitted. Genotype (G), GxN, and NxReplicates effects were considered as random and N and Replicates effects as fixed. The estimates of G and GxN effects were used for QTL mapping, as well as delta defined as the difference between GxN+ and GxN- estimates. The genetic map consisted of 3592 unique SNP locus obtained from the infinium 60K array and covered 2128.2 cM. QTL mapping was carried out using Multiple QTL Mapping (MQM) implemented in R/qtl package.

Results: For each trait, the genetic architecture consisted in a major QTL and few moderated QTL.The main QTL for DI-eH (Disease Index using eH) and DI-K92-16 were located on C9 and on the bottom of A3, respectively, these QTL showing epistatic interactions with the others QTL. N supply impacted DI QTL differently according to the isolates. The DI-eH QTL showed N modulation for most of QTL: The effect of C9-QTL decreased whereas the effect of C2 and C3 moderated-QTL increased under N limited conditions. For DI-K92-16 only one QTL for the GxN variable was identified on the bottom of A3. Moreover, a QTL can be N-responder for eH and non-responder for K92-16 as shown for the QTL on the top of A3.

Conclusions: The genetic control of clubroot resistance is modulated by N constraint, the effect of the main DI-QTL varying between the two N conditions. This modulation also depends on the *P. brassicaceae* isolates .

349
POSTERS THEME B

Epidemiology of sclerotinia stem rot of canola in New South Wales, Australia

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Background: Sclerotinia stem rot is a yield-limiting disease of canola caused by the necrotrophic fungal pathogen, *Sclerotinia sclerotiorum*. In Australia, the disease is prevalent in high-rainfall districts of southern New South Wales (NSW) where canola is frequently grown. Surveys conducted in Australia from 1998 – 2000 showed that the disease poses a threat to the Australian canola industry (Hind et al. 2003) and yield losses as high as 24% have been recorded under the Australian conditions (Hind-Lanoiselet and Lewington, 2004). The development of stem rot is highly sporadic due to its dependency on specific environmental conditions and inoculum levels. The only post sowing management tool currently available to producers is foliar fungicides. Therefore, a reliable forecasting system is needed to ensure that any in-crop foliar fungicide applications will be effective and economical.

Objectives: Understanding the epidemiology of sclerotinia stem rot in NSW is therefore paramount to assist in the strategic application of foliar fungicides. This can be done through identifying the 'trigger points' that lead to sclerotinia stem rot development by examining the interaction between environmental conditions, pathogen life cycle and the host.

Methods: Six commercial canola crops located in high disease risk districts of southern NSW were monitored for development of sclerotinia stem rot. Fifty petals collected from 50 different racemes separated one meter apart were collected from each crop and plated weekly to determine the level of petal infestation. Relative humidity and temperature at each crop were monitored using data loggers located in-crop, and rainfall data was taken from the nearest Bureau of Meteorology weather station. Measurement of the crop height, flowering stage and the presence of apothecia were recorded weekly. The types of infection (main stem, branch and leaf) were also recorded out of 100 plants within a crop to determine the level of stem rot incidence weekly.

Results: Four crops in NSW were found to develop significant levels of disease. Apothecia was scouted in all crops early before the commencement of flowering, therefore high levels of petal infestation (>90%) was detected upon flowering. Plants started to show symptoms of branch and stem infections commencing from the middle to late flowering period, which coincided with a prolonged period of high relative humidity (>90%) and rainfall events. Branch and stem infections increased up to approximately 40% and 30% respectively at some sites, even after the flowering period had finished and the relative humidity decreased.

Conclusions: Preliminary data showed that high initial inoculum levels, rainfall events and high relative humidity played a significant role in the development of stem rot during the flowering period. Once the flowering period had ended, branch and stem infections still progressed, mainly due to frequent rainfall events which enabled lodged infected senescent petals or leaves to cause further infection.

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Study of sclerotinia stem rot caused by *Sclerotinia sclerotiorum* on pre-flowering plants of *Brassica napus* in sichuan province of China

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Background: Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* is the most devastating disease on oilseed rape (*Brassica napus*) in China. Previous observations showed that in most cases, epidemics of oilseed rape SSR begin from ascospore-contaminated flower petals, which cause infection on leaves and stems when they fall on leaves and stems. In late December of every year since 2011, a kind of white mould disease on the stem base of oilseed rape plants at the bolting stage was observed in oilseed rape fields in Chengdu, Meishan and Guanghan County of Sichuan Province, China. The disease started by formation of water-soaked lesions, which spread rapidly on stems, leaf petioles and leaves. White cottony mycelia and black-colored sclerotia (6 mm in diameter on average) were produced on the lesion surface.

Objectives: The symptoms were very similar to sclerotinia stem rot caused by *Sclerotinia sclerotiorum*. This study is focus to disease type and disease incidence on Pre-flowering plants of *Brassica napus* in Sichuan province of China.

Methods and results: The diseased plants usually showed wilting appearance. *Sclerotinia* collected from diseased plants were, surface-sterilized for 5 min in 5% NaOCl, rinsed in sterilized water, placed on potato dextrose agar (PDA) and incubated at 20°C for 5 days. A total of 6 single-sclerotium isolates were obtained. All these isolates grew rapidly at 20°C on PDA and formed white-cottony mycelia and black-colored sclerotia. The morpho-cultural characteristics of these isolates appeared similar to *S. sclerotiorum*. Strain CanSS-QM1 was used for further identification by analysis of the ITS sequence and by specific PCR detection. The ITS sequence was cloned by PCR using the genomic DNA from CanSS-QM1 as template and ITS1/ITS4 as primers. The sequence (GenBank Acc. No. KC748491) showed 100% identity to that of *S. sclerotiorum* strain Ep-1PB (GenBank Acc. No. GQ404793). In the specific PCR detection, a 292-bp DNA fragment specific for *S. sclerotiorum* was obtained in nested PCR using two primers ITS4/ITS5 and XJJ21/XJJ222. This confirms the above-mentioned morpho-cultural identification. Pathogenicity was determined by placing mycelia of the strain CanSS-QM1 on detached leaves of *B. napus* cv. Chuanyou 58 at 20°C under humid conditions for 72 h. Necrotic lesions were produced from each inoculant and a fungus similar to strain CanSS-QM1 in morpho-cultural characteristics was re-isolated. The disease incidence varied in different fields and years: 5~85% in 2011 and 1~11% in 2012. Meanwhile, this disease was also observed in Sichuan on the stem base of a few cruciferous vegetables and weeds, including *B. juncea* var. *crassicaulis, B. compestris* var. *Purpurea*, *Capsella bursa-pastoris* and *Cardamine hirsuta L.*, with the incidence of 13% to 39%.

Conclusions: It shows that SSR caused by *S. sclerotiorum* on pre-flowering plants of oilseed rape in Sichuan province is increasingly widespread.

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351
POSTERS THEME B

Field effect of the seed-coating on clubroot of oilseed rape

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Background: Sichuan Province is the major oilseed rape production base in China, perennial planting area of about one million hectares. In recent years, the clubroot breaks out and spreads out on a large scale and the stricken area comes to 10~20 thousand hectares. The lost of production in serious area reached more than 80%, which is a great threat to the local oilseed rape production. Consequently, an effective control technology is urgent need.

Objectives: With the purpose of effectively controlling for clubroot of oilseed rape, we tried various studies on reagent combination, and then screened out BY1 seed coating formula. After that, we performed field efficacy experiments by seed-coating for clubroot on oilseed rape during 2013 and 2014.

Methods: We made the coating operation according to the weight ratio of 1:50. Lately on October 5, 2013 we conducted the field contrast test in the serous stricken area of Guanghai City (located in the central of Sichuan Province). Before direct seeding, the soil was ploughed, and the seeding rate was 2.5 Kg/ha. The seeding machinery is supported by Jingyang agricultural machinery cooperation. We used the seeds without coating as the contrast and the experimental plot area is 300 square meters. The repeated trials were conducted 4 times. The mature oilseed rape was reaped by harvester. Seed moisture determination was measured by the moisture test apparatus, and then dry weight under the 10% moisture content condition was calculated.

Results and Conclusions: After seeding 15d, we found that the seedling rate of coated seeds was 50~65 plants per square meter, while the contrast had 30~35 plants per square meter. The production of oilseed rape which treated with seed-coating was 2.465 t/ha, while the contrast had 1.442 t/ha. The results showed that production increased 1.023 t/ha and the growth rate reached to 71.4% in some serious plots, which indicated that the BY1 had great control effect for clubroot on oilseed rape. In other words, seed coating BY1 could significantly increase the emergence rate, survival rate and could decrease the damage of the clubroot.

Building a new defense line against *Sclerotinia sclerotiorum* infection

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Background: Stem rot caused by *Sclerotinia sclerotiorum* is a major yield-limiting factor in canola production. There is great variation between oilseed rape varieties and thus the resistance has become a major measure for control of this disease in China. There is however no complete immunity found in host plants, and the pathogen can fully infect plant (Liu et al. 2005) in varieties with the highest resistance, e.g. under high disease pressure in field. Thus there is an urgent need to develop new resistant varieties with significantly higher resistance against the pathogen.

Objectives: This study is to develop a new strategy that can significantly defense the pathogen infection on leaves and stem.

Methods: A comprehensive approach was developed to construct this strategy where high throughput genomics technologies were employed.

Results: The core idea is based on that the majority of disease lesions on leaves, stems and pods come from petal-mediated infection, and high and specific expression of foreign genes in petals will not have negative effect on plant development and avoids concern on effect of transgenic products on food chains, allowing us to use petals as a bioreactor to produce potent antifungal agents. The new strategy includes multiple components: highly efficient and short antimicrobial peptides (AMPs), highly specific and strong petal promoter with sustainable expression activity to sustain a large amount of AMPs in petals, and elements of anti-degradation of expressed proteins. We have screened all relevant genomic databases of animal, microbes and plants and predicted a large number of putative AMP genes. To experimentally test activity of candidate genes, we have established a set of high throughput methods, i.e. using the overlapping PCR to synthesize AMPs which are subsequently cloned into pET30a - EDDIE - GFP expression vector developed to express His-EDDIE-AMP fusion proteins in *Escherichia coli*, purification of the inclusion bodies and self-cleavage. We have obtained several recombinant AMPs with high antimicrobial activity. The above mentioned promoter was identified through in-depth RNA-seq of different tissues and subsequent function analysis. To increase more efficient inhibition on the fungus, multiple AMP genes were expressed together in a tandem array in which individual AMP release was allowed through enzymatic cut. After assembling these components, transgenic petals showed very high resistance in the model *Arabidopsis*. And transgenic *B. napus* is being tested.

Conclusions: The method based on bioinformatics tools and the described vector-screening is useful and high-throughput for discovery of AMPs. This new strategy is expected to efficiently complement naturally immune resistance bred through traditional or marker-aided selection to build two defense lines and thus efficiently prevent infection from the pathogen in oilseed rape.

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353
POSTERS THEME B

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Defining the molecular recognition of *Leptosphaeria maculans* effector *AvrLm1* by *Brassica napus* disease resistance protein LepR3

Background: The fungus *Leptosphaeria maculans* is the causal agent of blackleg posing a major threat to canola/oilseed rape (*Brassica napus*) worldwide. In *Brassica-L. maculans* pathogen system, 16 R genes that confer race-specific resistance to blackleg have been identified, and two of them, *LepR3* and *Rlm2,* have been recently cloned (Larkan et al., 2013; Larkan et al., 2015). Genetically, *LepR3* recognises *AvrLm1* and *Rlm2* recognises the recently cloned *AvrLm2* gene (Ghanbarnia et al., 2014). Recognition of Avr protein by its cognate R protein often culminates into a hypersensitive response (HR) around the infection site. Nicotiana benthamiana have served as a model plant to study the function of R/Avr proteins by *Agrobacterium* mediated transient expression.

Objectives: We exploited the *N. benthamiana* model plant to investigate recognition mechanism of *AvrLm1* by the recently discovered *LepR3* gene.

Methods: *Agrobacterium*-mediated transient expression was used to ectopically express AVR or R genes in *N. benthamiana.* Validation of protein-protein interaction was performed by co-immunoprecipitation and bimolecular fluorescence complementation (BiFC). Recombinant tobacco rattle virus (TRV)-based virus-induced gene silencing (VIGS) was used to silence the *Nbsobir1* in *N. benthamiana.*

Results: Co-expressing *LepR3/AvrLM1* gene pair in *N. benthamiana* results in development of HR observed in the infiltrated region. But, the truncated *AvrLm1* lacking indigenous signal peptide is unable to induce *LepR3*-mediated HR, indicating that *AvrLM1* is perceived by *LepR3* extracellularly. *LepR3* physically interacts with the *B. napus* receptor like kinase, *Bnsobir1*. Silencing of *Nbsorbir1* compromises *LepR3*-depended HR developments, suggesting that *LepR3*-mediated resistance to *L.maculans* in *B. napus* requires Sobir1. Structure-function analysis of *AvrLm1* protein reveals that C-terminal region is required for *LepR3*-mediated HR in tobacco and resistance to *L. maculans* in *B. napus.*

Conclusions: This is the first report on the use of tobacco as a model plant to study the function of R and Avr genes from *B.napus-L.maculans* pathosystem. Using this model system we were able to rapidly determine *Bnsobir1* as an essential partner of *LepR3* signalling complex and to define the *AvrLm1* effector domain. We also showed that in *Sobir1* silenced tobacco plants, *LepR3* failed to induce HR in response to *AvrLm1,* providing additional support for the role of *Sobir1* in *LepR3* mediated defence response.

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Growth promoting ability of *Trichoderma spp.* isolates on rapeseed

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Background: *Trichoderma* species have world-wide distribution in different types of soil. In addition to their antagonistic effect on a vast number of soil pathogens, *Trichoderma* species are known for their good growth promoting effects on numerous crops (Asaduzzaman et al. 2010).

Objectives: The aim of this study was to determine the ability of Serbian *Trichoderma* spp. isolates to promote the growth of rapeseed.

Methods: This study used ten *Trichoderma* spp. isolates originating from different soil types and localities in Vojvodina (Serbia). All isolates were refined to single-spore isolates for further research.The growth promoting activity was tested on 100 rapeseed seeds (cv. Zlatna) treated with *Trichoderma* spp. suspensions (2.5x106) for 30 minutes according to modified Mukhtar et al. (2012) method. Seeds with Trichoderma coating were dried at room temperature for 24 hours and germinated on double wet filter paper under optimal laboratory conditions. Seeds treated with sterile distilled water were used as control. Germination energy and germination was calculated on days 5 and 7 respectively, as a percentage of germinated seeds. Vigor index was calculated according to formula by Asaduzzaman et al. (2010). On day 7 root and shoot weight and length were also measured. All obtained data were analyzed in Statistica 10 by Duncan test (percentages were previously transformed in ArcSin√%).

Results: Seven out of ten tested *Trichoderma* isolates showed significant positive effect on at least one measured parameter of rapeseed seedlings. The isolate K176 can be singled out as the most effective, with the significant increase of five measured parameters – shoots length and weight, germination energy, germination and vigor index. Isolates K178 and K179 significantly increased root length and weight as well as vigor index of seedlings. Isolates K173, K174 and K175 significantly increased shoot lengths and weights, germination energy and germination, while isolate K150 significantly increased only seedling root weight.

Conclusions: Seven out of ten tested *Trichoderma* isolates expressed good growth promoting ability on rapeseed and those isolates should be further tested in more comprehensive research under the greenhouse and field conditions.

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355
POSTERS THEME B

Biological efficacy of *Trichoderma spp.* isolates against *Sclerotinia sclerotiorum* on rapeseed

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Background: *Trichoderma* species occur in soils and plant organic matters worldwide, and are well known as effective antagonists to a variety of soil fungal pathogens. *Sclerotinia sclerotiorum* is a cosmopolitan pathogen that colonizes over 400 plant hosts including oilseed crops, and can be efficiently controlled by *Trichoderma* spp.

Objectives: Due to good preliminary results in dual culture tests which indicated antagonistic activity of Serbian *Trichoderma* spp. isolates (Tančić et al. 2014), the aim of this research was to test the ability of those *Trichoderma* spp. isolates to protect rapeseed seedlings from *S. sclerotiorum.*

Methods: The study used ten *Trichoderma* spp. isolates from different soil types and localities in Vojvodina province (Serbia), and one *Sclerotinia sclerotiorum* isolate from sunflower grown at Rimski Šančevi, near Novi Sad (Serbia). The biological efficacy was tested on 100 rapeseed seeds (cv. Zlatna) treated with *Trichoderma* spp. suspensions (1x106) for 30 minutes according to modified Mukhtar et al. (2012) method. *Trichoderma*-coated seeds were dried at room temperature for 24 hours and placed in four replicates on wet double filter paper in Petri dishes. Next to each seed the 3 mm plug of *S. sclerotiorum* mycelia was placed. Seeds treated with sterile distilled water with no pathogen were used as a negative control, and seeds treated with sterile distilled water with the pathogen plugs were used as a positive control. Seeds were incubated under the optimal laboratory conditions. On day 7 biological efficacy of *Trichoderma* spp. isolates was estimated and calculated according to Liu et al. (2009). Data were analyzed in Statistica 10 by Duncan test (percentages were previously transformed in ArcSin√%).

Results: Biological efficacy of all tested *Trichoderma* isolates was statistically significant as compared to the positive control. Good antagonism with over 50% biological efficacywas registered in 6 isolates (K150, K160, K173, K176, K178, and K179). Germination was significantly higher in all treatments compared to the positive control. In 7 treatments germination was at the same level of significance as germination in negative control. Two treatments even had higher germination than negative control but not significantly different. Lower germination rates were mostly connected with lower biological efficacy of the isolates. The exception was the isolate K114 with low biological efficacy and high germination, which indicated a good growth promoting effect of the isolate but low protection from the pathogen.

Conclusions: Six *Trichoderma* isolates which showed biological efficacy over 50% should be included in further more comprehensive research.

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Decline in the concentration of resting spores of *Plasmodiophora brassicae* following a susceptible crop

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Background: The concentration of resting spores of *Plasmodiophora brassicae* Woronin in soil increases rapidly when susceptible crops are grown in short rotation. Some of these spores survive for many years (Wallenhammar 1996), but the rate of decline of resting spores in soil is unknown. However, a recent study indicates that there may be a practical advantage to a moderate (2- to 3-yr) break between a susceptible cultivar of canola (*Brassica napus L.*) and a clubroot-resistant cultivar (Peng et al. 2013).

Objective: To monitor the survival of resting spores for up to 6 years following a susceptible canola crop.

Methods: Small blocks (8 x 30 m) in a field infested with *P. brassicae* at the AAFC research site at Normandin, Québec, have been in cropping rotations over many years that includes clubroot susceptible canola. Resting spore populations in soil were quantified after continuous canola and break intervals of 1, 2, 3, 5, and 6 years between canola crops. Two blocks were selected for each length of break (0 to 6 year). Each block was divided into two parts, and five soil cores were collected and bulked within each part to form a representative sample. The concentration of resting spores in each sample was assessed using a multiplex qPCR protocol with an internal control (Deora et al. 2015). Three biological and three technical replicates were conducted for each sample. Log transformation, ANOVA, single df contrasts, and regression were used to assess the change in spore concentration over time.

Results: The concentration of resting spores in soil declined over time as the length of break from the susceptible canola crop increased. Regression analysis indicated a quadratic relationship of y = 1E + 07e-0.759x . R2=0.65. Compared to continuous canola (1.3 x 108 spores g-1 soil), resting spore concentration declined by 96% after a 1-yr break, 99% after a 2-yr break from canola, but then declined very slowly.

Conclusions: These results support a previous report (Peng et al. 2013) that large numbers of resting spores die or disappear in the first 1 to 2 years after a susceptible crop, but that some resting spores are persistent and may survive for many years. This observation has important implications for the importance of cropping rotations in management of clubroot on canola on the Canadian prairies.

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357
POSTERS THEME B

Why is clubroot a problem on canola in Alberta but not in Ontario, Canada

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Background: Clubroot, caused by *Plasmodiophora brassicae*, has been a major disease of canola in the province of Alberta since pathotype 3 (P3) was identified on that crop in 2003. The disease spread rapidly and has caused total crop losses in some fields. In contrast, clubroot has caused losses on vegetable crops in Ontario since the 1930's (Reyes et al. 1974) but does not appear to be a problem on canola.

Objective: To investigate the susceptibility of canola to *P. brassicae* at a high organic matters soil in Ontario and examine other factors that may affect the spread of clubroot in Ontario relative to Alberta.

Methods: Field trials were conducted in 2011, 2012, and 2014 at the Holland Marsh, Ontario, Canada at a site naturally infested with P6. There were 33 canola lines / cultivars tested in 2011, 13 in 2012, and 22 in 2014. Each trial included the clubroot-susceptible line ACSN39 as a control. The trials were arranged in a RCBD with four replicates. Clubroot symptoms were assessed on up to 50 plants per replicate using a 03 scale, and a disease severity index (DSI) was calculated. Cultivars were designated as moderately resistant if they had DSI values less than 30 and resistant if values were ≤ 10.

Results: High levels of clubroot developed each year on the susceptible line ACSN39 (66, 100, and 71 DSI, respectively). In 2011, 22 of 33 lines had a DSI < 30, and 12 of these had a DSI < 10. In 2012, 3 of 13 lines were classified as resistant and 7 of 22 lines in 2014. All of the cultivars that were resistant to P3 were also resistant to P6, but lines resistant to P6 were generally susceptible to P3 (data not shown). Canola is often grown in a 2-year rotation in Alberta, but crop rotations are generally much longer in Ontario, because the acreage is lower. There were only 14,000 hectares of canola in Ontario in 2014, compared to almost 2.6 million hectares in Alberta. Soil pH is similar in both provinces, but soils in Ontario may have higher levels of calcium. Canola may be seeded earlier in the spring in Ontario and winter canola is sometimes grown. Plant growth when temperatures are cool (< 15 oC) supresses clubroot development (Sharma et al 2011b).

Conclusions: The low level of clubroot on canola in Ontario can be attributed, in part, to the resistance of many canola cultivars to P6. Even cv. Westar, which is routinely used as a susceptible control, was partially resistant. Long cropping rotations and early spring or fall seeding may also suppress the establishment of *P. brassicae* in Ontario soils.

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Abundance of cabbage stem weevil, *Ceutorhynchus pallidactylus*, and rape stem weevil, *C. napi*, in northern Serbia

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Background: Expected increase of the acreage under rapeseed puts more pressure on insect control issues. Two weevil species, *Ceutorhynchus pallidactylus* Marsh., cabbage stem weevil, and *C. napi* Gyll., rape stem weevil, are the largest potential pest threats in Serbia.

Objectives: Stem weevils damage rapeseed crop proportionally to their abundance. Weevils presence and activity can be monitored using different methods, in order to obtain data on flight activity, population growth and date of maximum flight. Such information can be a valuable tool in estimating the necessity of chemical control. Furthermore, by combining different monitoring methods an accurate estimate of the number of weevils in the field can be obtained.

Methods: The monitoring of these pests was conducted in the vicinity of Crvenka, northern Serbia (N 45° 39′ 24.6″, E 19° 30′ 57.8″). Assessments were made every week during spring in 2011, 2012 and 2013, using three different methods, yellow water traps (Moericke dishes), entomological net (sweep net) and visual method. For this purpose four yellow traps were used. Sweep net was used at four different points in one field with 25 sweeps for each place. Visual assessments were made on 25 plants near each water trap. Collected specimens were identified in entomological laboratories at the Faculty of Science in Kragujevac and Institute of Field and Vegetable Crops using these keys: Alonso-Zarazaga 2004; Angelov 1979; Freude et al. 1983.

Results: Flight dynamics showed that the highest abundance of both species was observed in late March. Afterwards, in early April, the number of collected specimens rapidly decreased which can partially be explained by chemical control of pollen beetle carried out during the first week of April. Later in the season only individual specimens were sampled. Rape stem weevil (316 specimens) was three times more numerous than cabbage stem weevil (114 specimens). Regarding rape stem weevil sampling methods efficacy, yellow water traps were the most effective (282 specimens), followed by the visual method (51 specimens) and sweep net (28). In case of cabbage stem weevil sampling methods efficacy, the sweep net method was the most efficient (59 specimens), followed by yellow dishes (39 specimens) and the visual method (16 specimens).

Conclusions: Both species overwinter as adults and the beginning of rapeseed growing season coincides with the beginning of stem weevils' flight, which is usually early March in Serbia. It is advised to use a combination of methods to obtain better insight into the abundance of these harmful insects and their control, while the best results can be obtained with yellow water traps and visual method.

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359
POSTERS THEME B

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Pathogenicity of *Leptosphaeria maculans* isolates obtained from *Brassica napus* (oilseed rape) cultivars with the *Rlm7* resistance gene

Background: *Leptosphaeria maculans* causes phoma stem canker disease on oilseed rape (*Brassica napus*) (Fitt et al., 2006). The disease results in yield losses worldwide and causes losses to farmers of more than £100M p.a. at a price of £370 per tonne in the UK. A cost-effective and environmentally friendly method for control of the disease is the deployment of cultivars with resistance against *L. maculans*. Resistance against *L. maculans* involves both major (R) and minor resistance genes (Delourme et al., 2006). Major gene resistance operates on a "gene-for-gene" concept.

Objectives: To examine pathogenicity of *L. maculans* isolates obtained from cultivars with the resistance gene *Rlm7* on different *Rlm7* cultivars in controlled environment conditions in order to investigate differences between cultivars with the same R gene.

Methods: Isolates obtained from winter oilseed rape cultivars with the resistance gene *Rlm7* were examined for their pathogenicity by inoculation onto cotyledons or true leaves of the susceptible cultivar Drakkar (no R gene against *L. maculans*) and cultivars with the resistance gene *Rlm7* (Excel, Roxet, Hearty and line 01-23-2-1). After assessment of lesions, cotyledons and true leaves were detached 17 and 21 dpi, respectively, and incubated in darkness under high humidity to assess pycnidial development and conidial production.

Results: All the isolates tested on Drakkar produced typical large/grey lesions (susceptible phenotype) on both cotyledons and true leaves; large numbers of pycnidia with conidial masses were produced within and outside the lesions on cotyledons after 5 days of incubation and on true leaves after 3 days of incubation. All the isolates tested on the four *Rlm7* cultivars produced small lesions surrounded by dark margins (resistant phenotype) on cotyledons with no difference in lesion area between isolates. However, there were differences between isolates on true leaves of Roxet but not on true leaves of Excel, Hearty and 01-23-2-1. Most of the isolates produced small numbers of immature pycnidia (without conidial masses) on the lesions on cotyledons after 5 days of incubation and on true leaves after 3 days of incubation of the four cultivars with the *Rlm7* gene. Further incubation, when cotyledons and true leaves senesced, induced mature pyncidial development outside the lesions with conidial masses.

Conclusions: Different cultivars with the *Rlm7* gene may respond differently following inoculation with *L. maculans* isolates in controlled environment conditions. Asexual reproduction can take place on resistant cultivars when the tissue senesces.

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Phoma stem canker on oilseed rape cultivars with good resistance against *Leptosphaeria maculans*

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Background: Phoma stem canker, caused by the related pathogens *Leptosphaeria maculans* and *L. biglobosa,* is an economically important disease of oilseed rape (*Brassica napus*) worldwide (Fitt et al., 2006). There is a 'gene-for-gene' interaction between *L. maculans* and *B. napus* at the leaf spot stage of the disease. It has been suggested that one of the resistance genes that has been widely deployed in new oilseed rape cultivars across Europe, Rlm7, is more durable than other commercially available R genes (Clarke et al., 2011). Monitoring phoma stem canker severity in crops and the frequency of the virulent isolates in pathogen populations can help to predict increases in pathogen virulence and to manage the risk of severe disease epidemics.

Objectives: To examine the *Leptosphaeria* populations on Rlm7 cultivars in the UK, to investigate emergence of isolates virulent against Rlm7 and to determine molecular mechanisms leading to virulence. To examine the importance of the co-existing pathogen *L. biglobosa* in determining severity of phoma stem canker on cultivars with the Rlm7 gene.

Methods: Leaves with phoma leaf spots were sampled from cultivars carrying the Rlm7 gene and a cultivar with no known R genes (Drakkar) in autumn/winter the UK (2011/2012, 2012/2013 2013/2014 cropping seasons). Severity of phoma leaf spotting caused by *L. maculans* or *L. biglobosa* was assessed. Single pycnidial isolates were obtained and pathogen identification was done by observations on PDA and species specific PCR. Frequencies of the avirulent AvrLm2, AvrLm3, AvrLm4 and AvrLm7 alleles in *L. maculans* populations were investigated at different sites in the UK by inoculation on cotyledons of cultivars with the corresponding Rlm genes.

Results: There were differences in severity of phoma leaf spotting on Rlm7 cultivars between seasons. The number of *L. maculans* leaf spots on Rlm7 cultivars increased from 2011/2012 to 2012/2013. Phoma leaf spotting caused by *L. biglobosa* was more severe on the Rlm7 cultivars than on Drakkar. All *L. maculans* isolates obtained in 2011/2012 were virulent against Rlm2 and Rlm3 and avirulent against Rlm7. Isolates virulent against Rlm7 that were found in 2012/2013 were avirulent against Rlm3. Isolates virulent against Rlm4 were present in the *L. maculans* populations.

Conclusions: Breeding for resistance against *L. maculans* may affect susceptibility of cultivars to *L. biglobosa*. There may be opportunities for sequential deployment of different R genes in oilseed rape crops.

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361
POSTERS THEME B

Molecular characterization and identification of fungi causing rapeseed stem canker in Serbia

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Background: During the 20th century the pathogenic fungus *Leptosphaeria maculans* was on the quarantine list in Serbia. The pathogen was first isolated in Serbia in 1995 from seed and consume cabbage, and in 1987/88 from rapeseed. Cultivation of rapeseed intensified towards the end of the 20th century, and based on the examination of production fields during 2005 and 2006, parasitic fungus *Leptosphaeria maculans* was isolated in all production areas of the Province of Vojvodina, Serbia. More frequent symptoms on the aboveground plant organs lead to the conclusion that this parasite on rapeseed might become economically important in the future.

Objectives: The aim of this study was to identify pathogenic fungi causing stem cancer in rapeseed in Serbia by using molecular methods.

Methods: Stem canker of rapeseed is economically important disease in Europe, Australia and North America. This disease is caused by two species: *Leptosphaeria maculans* (Desm.) Ces. et de Not (*anamorph Phoma lingam* (Tode ex Fr.) Desm. and *Leptosphaeria biglobosa Shoem.* and *Brun. B. napus* plant material infected with *L. maculans* and *L. biglobosa* was collected from nine sites in Serbia (Karavukovo, Crvenka, Prigrevica, Subotica, Rimski šančevi, Srbobran, Beška, Banatsko Karađorđevo, Srpski Miletić). Infected tissue samples were taken in 2008-2010 from root, upper and basal stems, leaf, flower and pods. Total of 119 isolates from Serbia and two from Great Britain were analyzed using PCR and PCR-RFLP. Digestion of PCR products was performed with 5 selected endonucleases: BamHI, HaeIII, Rsa1, EcoRII i Alu1. From a total of 119 isolates originating from Serbia and two representative isolates, 15 isolates were used for PCR-RFLP analysis.

Results: Based on DNA amplification with PN3 and PN10 primers, band length was 580 bp in isolates K-111 to K-118, and 560 bp in the remaining isolates (K-1 to K-25, St-1 to St-28, GS-1 to GS-27, C-1 to C-6, L-1 to L-10, S-1 to S-11, LJ-1 to LJ-6). PCR-RFLP analysis showed that K-111, K-112, K-113, K-115 and K-116 differed in one or two restriction locations from isolates K-2, St-16, GS-25, L-5, C-3, LJ-2, and S-1.

Conclusion: Based on the PCR analysis of all isolates originating from Serbia, it was determined that 111 belong to *Leptosphaeria maculans* and 8 to *Leptosphaeria biglobosa* NA1 (*Leptosphaeria biglobosa brassicae*)

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Influence of timing of volunteer oilseed rape control on clubroot disease development and inoculum density of *Plasmodiophora brassica*

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Background: Clubroot of oilseed rape (OSR; *Brassica napus*) caused by *Plasmodiophora brassicae* Woronin is a disease of increasing importance in OSR growing regions worldwide. The disease is difficult to control and disease management depends mostly on resistant cultivars, long crop rotation and the soil alkalization.

Objectives: Till date, any fungicide has been discovered to give complete control over clubroot and presently, the number of resistant cultivars is limited. Previous studies indicated that clubroot severity on OSR increases with increasing density of *P. brassicae* inocula (Hwang et al. 2011). Besides, it was shown that OSR volunteers and weeds play a critical role in development of clubroot epidemics by increasing the number of resting spore populations in the soil (Murakami et al. 2002; Ahmed et al. 2011). Therefore, it is necessary to study and develop management strategies for decreasing inoculum density in the soil.

Methods: A series of experiments were conducted under controlled glasshouse conditions with a susceptible OSR variety to clubroot to assay the effect of timing of foliar application of the herbicide glyphosate and mechanical destruction of OSR volunteers in reducing inoculum density and subsequent clubroot disease severity. Oilseed rape plants were inoculated artificially with injecting spore suspension (2*107 spores/ml) beside root hairs at BBCH 11-12. To determine the effect of timing of applications, plants were terminated early (7 dpi) or late (21 dpi). The amount of disease severity and the number of resting spores per gram of root was assessed for each treatment at 35 dpi. Later on, according to the Koch's postulate test, the effect of early or late volunteers and weeds destruction on pathogenicity factor of new resting spores was evaluated on new set of OSR plants.

Results: Visual disease assessments after early and late applications showed well variation among the treatments in symptom development. Changing the time of application had significant effect ($P \leq 0.05$) on control efficiency. Results from this study demonstrated that the early application of glyphosate as well as the early mechanical destruction significantly decreased, relative to untreated control, the development of clubroot symptoms and suppressed the establishment and survival of the resting spores. Afterwards, the disease incidence and severity were significantly decreased in new plants which inoculated with the spore extraction from early treated roots.

Conclusions: Our results described that early treatment of OSR volunteers and weeds reduced significantly symptom severity and spore production.

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363
POSTERS THEME B

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Life history of the rape pollen beetle, *Brassicogethes viridescens*, in a canola crop on Prince Edward Island

Background: The rape pollen beetle, *Brassicogethes viridescens*, an introduced species to eastern North America from Europe, is causing damage to local *Brassicaceae* plants, including canola (*Brassica napus*). With a small canola industry, ~3000 acres in PEI annually, eastern Canada has a low potential for economic loss due to this species. However, this species is projected to migrate further west into the prairie ecozone of Canada where ~10,000,000 acres of canola are grown annually (Mason et al., 2003); creating a high potential for economic loss.

Objectives: The key objectives of this study include: (1) Determining time periods and relative degree-days for several life cycle stages of *B. viridescens* relative to *B. napus* phenology, and comparing *B. viridescens* life cycle time periods on PEI to those in central Europe. (2) Determining the damage caused by *B. viridescens* feeding and oviposition in canola buds on PEI.

Methods: The study was conducted at Agriculture and Agri-Food Canada's Harrington Research Farm, PEI [46.343/-63.164] in 2014. Several trapping techniques were used to collect various stages of *B. viridescens* during its life cycle within canola plots and the hedgerow. These techniques included sticky traps (to intercept emerging adults and new generation adults entering overwintering sites), manual collection of buds (to record eggs and larvae, and damage), pupation traps, and adult emergence traps. Degree days were calculated using the equation DD = (T°-T(t)) and the relationship between buds with damage and buds with young was tested using linear regression.

Results: The active period of *B. viridescens* life cycle (overwintered adult emergence to new generation entering overwintering sites) occurred between late June and early September in 2014 and required 799.2 DD above 10oC. Life cycle time periods were later in PEI compared to those reported from central Europe. Mating of *B. viridescens* adults and development of the young was found to coincide with plant phenology. A significant positive relationship was found between the number of buds with damage and the number of buds containing *B. viridescens* young ($p<0.001$).

Conclusion: Emergence of overwintered adults occurred in late June (average temperature: 16.15°C), much later than emergence in central Europe (late-April; average temperature: 5-10°C; Nilsson, 1989). This indicates that other factors are involved in the emergence of adults besides temperature, such as photoperiod, or adaptation by *B. viridescens* to *B. napus* phenology in North America. The degree-day values calculated in this study can be used to predict *B. viridescens* life stages in canola fields, estimate potential damage by relating percent damaged buds to the percent young within buds and time control applications more precisely.

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Mining QTL for candidate genes involved in resistance of oilseed rape against *Verticillium longisporum* by an integrative omics approach

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Background: *Verticillium longisporum* is an increasing threat to winter oilseed rape production in Europe. Resistance against *V. longisporum* is quantitatively inherited. Commercial European winter oilseed rape cultivars exhibit no or very low levels of quantitative tolerance or resistance. Quantitative trait loci have been identified in biparental crosses using resynthesized lines with the most effective partial resistance originating from the C genome of *Brassica oleracea*. Resistance against *V. longisporum* is expressed internally in the vascular tissue of the hypocotyl and phenolic compounds have been found to be associated with resistance expression (Eynck et al. 2009, Obermeier et al. 2013).

Objectives: The integration of data obtained from the genetic analysis of segregating populations and breeding material applying a combination of targeted and untargeted high-throughput omics-based technologies (genomics, metabolomics, transcriptomics) might allow more efficient identification of involved pathways and candidate genes than by any of these technologies alone. This integrative approach might speed up the development of broadly applicable diagnostic molecular markers for use in marker-assisted breeding for *V. longisporum* resistance.

Methods: A doubled haploid mapping population produced from an inbred line of a German rapeseed cultivar and a resynthesized rapeseed line segregating for *V. longisporum* resistance (Express617 x R53) was used for comparative genetic, genomic, transcriptomic and metabolomic analysis. The non-targeted analysis included the production of a high density genetic map using genotyping-by-sequencing and 60K SNP chip Illumina Infinium genotyping and quantitative trait locus (QTL) mapping for disease related traits and comparison with global RNA-Seq data. The targeted analysis included the identification and quantification of phenolic metabolites and lignin monomers in the hypocotyls of the mapping population by RP-HPLC and GC-MS to evaluate association with resistance expression and identify metabolite QTL and genes co-localizing with resistance QTL.

Results and Conclusions: Omics-based dissection and integration of metabolomic and genetic data from the mapping population with transcriptomic data obtained by RNA-Seq analysis from contrasting genotypes allowed efficient mining of QTL for candidate genes and pathways involved in resistance expression. This approach allowed to establish an improved understanding of the multiple mechanisms and pathways involved, rank them by relevance and prioritize candidate genes from the QTL interval. The contribution of different interconnected pathways and genes to resistance expression in the highly complex pathogen-host interaction will be discussed. Based on this approach kompetitive allele-specific PCR (KASP) markers have been derived and are being used by German oilseed rape breeders for marker-assisted selection.

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365
POSTERS THEME B

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Evaluation of seed treatments for control of leafhoppers and suppression of aster yellows in hybrid canola

Background: Aster yellows (AY) caused major yield losses to canola in western Canada in 2012 (Miller et al. 2013). The phytoplasma is transmitted by the aster leafhopper, *Macrosteles quadrilineatus* that is brought into western Canada on winds from the southern US in early spring. AY symptoms are more severe when canola seedlings are infected in wet soil than in dry soil (Olivier et al. 2014). AY is difficult to control. An application of dimethoate is the only method currently registered for leafhopper control in canola but no economic threshold has been established (Anonymous 2015). Neonicotinoid seed treatments provide effective leafhopper control at early seedling stages in other crops. The treatments are currently registered for flea beetle control on canola in Canada and have not been evaluated against leafhoppers.

Objectives: Assess the effect of soil moisture on the efficacy of neonicotinoid seed treatments for control of leafhoppers and suppression of aster yellows at different growth stages of hybrid canola.

Methods: Untreated seeds and seeds treated a fungicide (Tribune) or neonicotinoid seed treatment containing imidacloprid (Gaucho CS FL), clothianidin (Prosper Evergol) or thiamethoxam (Helix, Helix XTra) were grown in dry soil (20-30% moisture content) and wet soil (70-100% moisture content). Plants at the cotyledon and 1st-4th true-leaf stages were placed in cages and exposed to AY-infected leafhoppers (n = 6 adults/plant) at 20°C for 72 h. Leafhopper mortality was assessed after 24 and 72 h. Plants were grown at 20°C under high light intensity (>400 umol/m^2/s). AY symptoms were assessed after 6, 8 and 10 weeks using a five-point rating scale (Olivier et al. 2014). Plants were harvested at maturity to determine seed yield.

Results and Conclusions: Seed treatments and soil moisture had a significant effect ($P \leq 0.001$) on leafhopper mortality at the cotyledon and early true-leaf stages. At each growth stage, mortality in the neonicotinoid seed treatments was higher in dry soil than in wet soil. In dry soil, Prosper, Helix and Helix XTra provided excellent control of adult leafhoppers after 24 h at the cotyledon stage (93-96%), 1st & 2nd true-leaf stage (90-97%) and 3rd & 4th true-leaf stage (95-100%). Mortality in the treatments after 72 h exceeded 95% at each growth stage. Treatments were less effective in wet soil. Mortality with Prosper, Helix and Helix XTra in wet soil averaged 68-81% at the cotyledon stage, 65-74% at the 1st & 2nd true-leaf stage and 81-88% at the 3rd & 4th true-leaf stage. Mortality in the treatments after 72 h averaged 86-91%, 71-89% and 95-99%, respectively. AY symptoms and yield are currently being assessed. Preliminary results suggest that greater than 80% control of leafhoppers within 24 h may be required to prevent AY infection and yield loss in dry and wet soil.

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Distribution and characterization of clubroot pathotypes in the main WOSR production areas in France

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Background: Clubroot is an important soil-borne disease of *Brassica* crops caused by *Plasmodiophora brassicae,* an obligate biotrophic protist. The disease is characterized by the development of galls on the root system disturbing water and mineral nutrition of the plant, leading to premature death of the plant and causing significant yield losses. The pathogen can remain in the soil as viable spores during 20 years. Using resistant varieties is one of the best ways to control this disease in *Brassica napus* crops. To date, no official test exists for registration of clubroot resistant varieties to the French Official Catalogue of Plant Varieties.

Objectives: To determine the distribution and characterization of the *P. brassicae* pathotypes in the main *Brassica* production areas in France and to develop a protocol for evaluating the resistance to clubroot in WOSR varieties.

Methods: Location and characteristics of infested fields were monitored by CETIOM through an online reporting system (http://www.cetiom.fr/hernie/). Field sampling was done in Centre, Bourgogne, Bretagne, Lorraine and Poitou-Charentes regions in France, targeting different environmental situations. Biological characterization of *P. brassicae* field populations isolated from infected plant clubs was done using the differential host set defined by INRA of Rennes, allowing the identification of eight pathotypes (Some et al., 1996; Manzanares-Dauleux et al., 2001).

Results: A total 70 samples was analyzed. Pathotypes P1 to P6 were identified in the territory, P1, P2 and P3 being the most frequents. More than half of the isolates were identified as P1. Several isolates belonging to pathotypes P1, P2 and P3 were able to overcome the resistance of Mendel (reference resistant variety).

Conclusions: Isolates from the most frequent pathotypes in France (P1, P2 and P3) have been chosen in order to test the resistance of the WOSR varieties. A complete protocol to evaluate resistance has been proposed to the French registration committee. This protocol is now currently used by GEVES to evaluate resistance of WOSR varieties for registration.

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367
POSTERS THEME B

Twenty years of canola variety performance in the Pacific Northwest

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Background: The Pacific Northwest (PNW) has a long history of growing small grain cereal crops, such as wheat and barley. Only about 2% of land in the PNW that is suitable for cereal grain rotations is planted to canola. Canola is a good rotational crop when grown with wheat and barley and adds diversification to dry-land farmers of the PNW. Rotational benefits include improved control of pests, grass weeds, and diseases in cereal production. A major constraint on increasing canola acreage has been the availability of suitably adapted cultivars.

Methods: Researchers at the University of Idaho established the Pacific Northwest Spring and Winter Variety Trials in the early 1990s and have conducted winter and spring cultivar trials throughout the PNW region for the past 20 years, testing cultivars and breeding lines from the University of Idaho alongside entries from private and other public breeding programs. The trials were typically grown at four locations in Idaho, three in Washington, and two in Oregon.

Over the 20 years of testing, 266 spring cultivars from 25 different companies and 160 different winter varieties from 20 different companies or breeding programs have been tested. 'Westar' spring canola was included in all spring trials as a control, and 'Bridger' winter rapeseed was included in all winter trials as a control. Both of these controls were used as checks to determine the proportion of yield improvements that were attributable to advances in crop genetics.

Results: The yield of the three best winter canola cultivars increased from 3,400 kg ha-1 to over 4,400 kg ha-1, an increase of 52 kg ha-1 each year. The cultivar 'Bridger' showed a yield increase from 2,601 kg ha-1 to 3,070 kg ha-1, 23 kg ha-1 per year. This yield increase for Bridger can be attributed to improvements in agronomic practices, including new pesticides. Comparison of the genetic and non-genetic yield gains shows that winter canola genetic improvements contributed yield increases of 604 kg ha-1 (55%), while agronomic improvements contributed 483 kg ha-1 (45%).

Yield of the best cultivars entered into the spring variety trials have shown an improvement in yield from 1,950 kg ha-1 to over 2,500 kg ha-1. 'Westar' also showed a yield increase from 1,665 kg ha-1 to 1,844 kg ha-1. Comparing genetic with non-genetic yield gains, spring canola cultivars showed improvement due to genetics of 470 kg ha-1 (70%), while agronomic improvements accounted for an increase in yield of 188 kg ha-1 (30%).

Conclusions: Genetic improvements of cultivars and improved agronomic practices have increased yield potential for spring and winter canola significantly. In recent years, the acreage of canola in the PNW has risen and continues to increase. This is due in a large part to the availability of new and adapted cultivars in combination with improved agronomic practices.

368
POSTERS THEME B

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Transcriptome sequencing and gene networks analysis reveals pathogen effectors manipulate the JA and SA pathways of *Brassica napus*

Background: The white mold fungus *Sclerotinia sclerotiorum* is a devastating necrotrophic plant pathogen with a remarkably broad host range (Guyon et al. 2014), however, very few studies have investigated host-pathogen interactions about the genetics and biochemistry.

Objectives: Identification of gene networks relate to disease-resistance in *Brassica napus* or pathogenicity in *S. sclerotiorum* will help us understanding the mechanism of rape-*S. sclerotiorum* interaction and guide the commercial rapeseed breeding.

Methods: A uninfected leaf of 'NRS-1' together with hypha of 'S06SZ' were used as one sample. Three infected leaves and pathogenic mycelium collected at 6h, 24h and 48h, respectively, were used as the other three samples. All these four samples' total RNA were used for transcriptome sequencing, respectively.

Results: A total of 80.46M high-quality reads were obtained by Illumina HiSeqTM 2500 sequencing of the rape-*S. sclerotiorum* interactome. Tophat software was used to distinguish rape and *S. sclerotiorum* sequences. 52,159 rape unigenes and 13,313 *S. sclerotiorum* unigenes were predicted to be expressed specifically during the rape-*S. sclerotiorum i*nteraction. Among those unigenes expressed in 'NRS-1', 2,528 at 6h, 2,064 at 24h and 2,475 at 48h were detected as Differentially Expressed Genes (DEGs). The same method was also used to analyze *S. sclerotiorum* unigenes, and we found 698 DEGs, 475 DEGs and 540 DEGs, respectively. A method based on the BLAST program was modified to obtain DEGs annotation from Kyoto Encyclpedia of Genes and Genomes (KEGG) pathway database. At 6h, 'NRS-1' expressed 127 genes were annotated to ribosome assemble. Then at 24h, 10 genes were annotated to alpha-Linolenic acid metabolism and 48h, 35 genes were annotated to plant-pathogen interaction and indolylmethyl-glucosinolate biosynthesis were expressed specifically. All these pathways were related to jasmonic biosynthesis and signaling transfer. Additionally, Shikimic acid pathway, which was reported as another way of salicylic acid synthesis in plants, was found in *S. sclerotiorum* at 24h. The expression patterns of the 22 genes belong to these pathways were analyzed by RT-qPCR to explore their putative functions.

Conclusions: Our data reveals the different stage when *S. sclerotiorum* infecting, *B. napus* will activate defence genes against fungal infection and most of defence genes depend on the inducing by JA pathway. The reason that necrotrophic fungal pathogen *S. sclerotiorum* suppress JA-dependent defenses may caused by effectors which targeting inhibiting JA signaling but activating SA pathway in *Brassica napus*.

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369
POSTERS THEME B

Using genome-wide SNPs to facilitate the selection of genetic background for efficient introgression of clubroot resistance from diploid *Brassica rapa* vegetable species into amphidiploid *B. napus canola*

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Background: Diverse host resistance mechanisms help durable resistance for clubroot (*Plasmodiophora brassicae* Woronin) management on canola. Most clubroot resistance (CR) genes have been found in the diploid species *B. rapa* (2n = 20, AA), but >90% of canola cultivars in Canada are the amphidiploid *B. napus* (2n = 38, AACC). Interspecific hybridization can be used to move CR genes from *B. rapa* into *B. napus*, but most BC1 plants carry univalent C chromosomes resulted from unpaired C chromosomes from the triploid F1 (2n = 29, AAC). Additionally, varying amounts of undesirable background may be carried over from the CR donor. Normally repeated backcrossing is used to stabilize suitable chromosomal content and eliminate undesirable genetic background.

Objective: Use SNP markers to select plants with the most desired genetic composition at early stages of introgression to accelerate the development of CR canola germplasm.

Methods: Interspecific crosses were made between *B. rapa* ssp. *chinensis* 'Flower Nabana' (FN) carrying the CR gene Rcr1 (Chu et al. 2014) and *B. napus* line DH16516 originating from 'Topas'. Resistant F1 progenies were crossed further with the *B. napus* canola DH line SV11-17667 to produce a BC1F1 population. SSR flanking markers and a bioassay were used to select plants carrying Rcr1. The genetic composition of the A and C genomes were examined using an Infinium 6K SNP array. Plant development and yield potential were assessed for selected plants with varying genetic content and composition under controlled conditions and resulting seed were analyzed for fatty-acid profiles.

Results: Rcr1 was present in about half of the BC1F1 population. The background SNP analysis found that each of the resistant plants carried nine C genome chromosomes from SV11-17667, but 1 to 9 from DH16516, resulting in varying numbers of univalent C chromosomes in the BC1F1 plants. Additionally, some of the polymorphic A-genome SNPs that showed the same segregation pattern as in the parental line FN were found also in these BC1F1 plants, ranging from 22% to 64%. This potentially denotes varying amounts of genetic background carried over from FN. It was noteworthy that plant # 66, which carried Rcr1, a full complement of C-genome chromosomes and 40% of SNPs shared with FN, exhibited morphological characteristics and fatty-acid profile similar to those of SV11-17667. These yield/quality traits often were better than those observed for other resistant BC1F1 plants carrying 1 to 8 univalent C chromosomes.

Conclusions: Background selection using the SNP array allowed rapid identification of a desirable line in a population derived from the crosses between *B. rapa* and *B. napus* species, showing the potential of using the technology to accelerate CR introgression into *B. napus* canola.

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The profile of avirulence alleles in the population of *Leptosphaeria maculans* (blackleg) in western Canada

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Background: Blackleg disease of canola (*Brassica napus*), caused by the fungus *L. maculans* (Desmaz.) Ces. & de Not, has increased in western Canada in the past several years, with severe cases reported on R-rated canola cultivars. Resistance conferred by major-gene and quantitative (adult-plant resistance - APR) exists in Canadian canola cultivars, with >70% carrying Rlm3 and 56% with APR. Other specific resistance genes are uncommon. Recent analysis of *L. maculans* race structure in random field samples confirmed an earlier notion (Kutcher et al. 2007) that Rlm3 was no longer effective against blackleg in western Canada.

Objectives: To better understand the race structure and dynamics of *L. maculans* population in western Canada, and investigate potential adaption of the pathogen to resistant cultivars.

Methods: Approximately 50 trap plots/strips of cv. Westar (carrying no resistance genes) were seeded across canola-growing regions in western Canada in 2012 and 2013. Diseased stems were collected from 10-15 locations each year and up to 25 *L. maculans* isolates were tested from each location using host differentials carrying known resistance genes to profile Avr alleles in the pathogen population. Likewise, diseased samples from commercial fields with varying blackleg incidence/severity were also collected and *L. maculans* isolates tested. A total of 424 and 300 isolates from the trap plots and commercial fields, respectively, were tested using an established protocol (Kutcher et al. 2007).

Results: In trap plots, AvrLm1, AvrLm3, AvrLm9, AvrLep1 and AvrLep2 showed low frequencies (0-9%), whereas AvrLm2, AvrLm4, AvrLm6 and AvrLm7 were common (>70%), with AvrLm7 at 99% in each region by 2013. The low frequencies of AvrLep1 and AvrLep2 may be related to the test protocol used. The results indicate that the resistance genes Rlm2, Rlm4, Rlm6 and Rlm7 are generally effective against the current pathogen population. Some regional differences were observed: AvrLm4 was noticeably lower in Alberta (35%) than in Saskatchewan and Manitoba (>75%), while AvrLm2 was most common in Saskatchewan (100%) relative to the other two provinces (60-70%). In commercial fields, AvrLm1, AvrLm3, AvrLm9 and AvrLep2 were absent or at very low frequencies. Varying severity of blackleg in these fields, however, can't be explained by the pathogen race structure alone; AvrLm3 was generally missing in the pathogen population and only the resistance gene Rlm3 had been used in Canada. Likely APR is common in current canola cultivars, otherwise more severe blackleg could have occurred in many regions. Often the Avr profile in a severely diseased field did not differ substantially from that in other commercial fields or trap plots in the same region.

Conclusions: Analysis of Avr allele frequencies in the *L. maculans* population indicates that Rlm2, Rlm4, Rlm6 and Rlm7 are effective against the current pathogen population in western Canada. Varying severity of blackleg on R-rated cultivars may be caused by multiple factors, in addition to variations in Avr allele frequencies observed.

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371
POSTERS THEME B

Choice of cultivar – the key tool in oilseed rape disease control

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Background: Oilseed rape is an important crop in Lithuania in terms of the area grown and fungicide inputs. A high concentration of oilseed rape in a crop rotation in recent years and its frequent return to the same field have resulted in high pressure of the most important diseases such as sclerotinia, blackleg, black spot, verticillium wilt and others. Different disease management tools must be used giving priority to environmentally friendly measures and any fungicide use has to be balanced against the risks of losses to diseases.

Objectives: The aim of this study was to establish the resistance of different rapeseed cultivars (cvs.) to the main diseases in the natural infection conditions.

Methods: Field experiments were carried out during the 2013-2014 growing seasons with 21 cvs. of winter and 21 cvs. of spring oilseed rape, which represent a wide range of resistances and susceptibilities to diseases using predominantly cvs. available on the national market. Visby and Landmark, the currently most popular cultivars, were used as references for winter and spring oilseed rape, respectively. The experimental design included the 2 main plots: 1. natural infection, with no fungicide; 2. fungicide applied at GS 65. Disease pressure was established according to the assessment scales (Aubertot et al. 2004; Krüger, 1991). The seed yield of each cultivar was estimated.

Results: Sclerotinia, blackleg and verticillium dominated in the natural field conditions in winter and spring oilseed rape during the experimental year. Winter oilseed rape cv. Visby was the most resistant to blackleg; however, it was the most susceptible to sclerotinia. Cv. Cult OP was 2.4 times less damaged by sclerotinia compared to the reference Visby. Cv. Nelson H was the most susceptible to verticillium wilt. The best yield response to fungicide application at GS 65 was achieved in cv. Remy OP, lower response was in cv. Cult OP. Blackleg severity was 2.6-3.0 times higher in spring oilseed rape cvs. Lennon OP, Fenja OP and Majong H compared to the reference Landmark OP. Cv. Smilla H exhibited the highest resistance to sclerotinia. The highest verticillium wilt severity was recorded in cvs. Smilla H and Traper H, lower severity in cvs. Kaliber H and Fenja OP. The highest yield potential was shown by cvs. Lennon OP, Majong H, Fenja OP and Jegger OP.

Conclusion: Choice of cultivars with satisfactory disease resistance attributes can reduce the need for fungicide input.

Acknowledgements: The paper presents research findings, obtained through the long-term research programme "Harmful organisms in agro and forest ecosystems" implemented by Lithuanian Research Centre for Agriculture and Forestry.

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Levers of tolerance to floral bud damage in *Brassica napus*

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Background: Floral bud damage generated by pollen beetle is a source of dramatic nuisibility on the final crop yield (Lerin, 1987). However, nuisibility remains variable according to plant compensation that implies the number of fertile branches, the number of pods, and the pod weight (Tommey and Evans, 1992; Pinet, 2015).

Objectives: Objective of the study is to assess the respective implication of the key levers cited above in the compensation in seed yield. Therefore, damage of varying intensities and different modalities (floral bud or inflorescence clipping) has been applied to plant with varying branching potential generated by the interaction between genotypes and nitrogen fertilisation levels.

Methods: Three varieties with contrasting architectures were studied during two years (2007 and 2008) combined to two levels of nitrogen fertilization (high: 140 and 150kg.ha-1 for year 1 and year 2; and low: 70 and 40kg.ha-1 for year 1 and year 2). Three intensities of inflorescence clippings were applied in year 1 on five plants before flowering (5% to 50% of floral buds destroyed according to treatments). Three intensities of floral bud clipping were applied in year 2 on 13 plants (23% to 94% of floral bud destroyed according to treatments). At harvest the number of pods, axes, and pod per axis were counted, the dry mass of different compartments of the plant was weighted on plant sample of varying sizes according to years and treatments.

Results: Results are that the major lever of compensation of the damage in terms of seed yield was the number of pods, before the pod weight. Primary branches present prior to clipping mainly carried the compensating pods, and the proportion of yield beard by the secondary branches was increased. A correlation between the compensation in seed yield and the number of fertile axes was evidenced. Variations in branching and biomass allocation were observed between the clipping modalities that can be explained by the fact that floral bud clipping did not affected the vegetative mass of the plant while the clipping of the whole inflorescence did.

Conclusions: Reproductive morphogenesis is a key process of plant answer to floral bud damage. This study helps understanding plant tolerance to pollen beetle on *Brassica napus*. A dynamics and spatial study on pod setting on the plant would help refine the mechanisms involved and their genotypic variability (Pinet, 2010).

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373
POSTERS THEME B

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Evolution of *Leptosphaeria maculans* populations in a small area of the region Centre (France) following the introduction of oilseed rape hybrids carrying the *Rlm7* specific resistance gene

Background: The specific resistance to blackleg conferred by *Rlm7* has been used in commercial oilseed rape (*Brassica napus*) cultivars since 2004. Varieties carrying *Rlm7* have since become widespread today with a very large market share at the national level. In order to evaluate consequences of the selection pressure exerted by the *Rlm7* resistance gene on populations of *Leptosphaeria maculans* (causal agent of blackleg), samples of isolates of *L. maculans* were collected for ten years on plants both with and without *Rlm7* in a small area in the central region of France.

Objectives: The main objectives are:

1. To improve tools to be able to follow qualitative evolution of fungus populations.

2. Follow *L. maculans* population evolution over a long period in the centre of France, which is a production area where the disease risk is important.

Methodology: Each year from 2004, sampling was done in 20-30 different fields in late November or early December. One leaf per plant and one isolate from a blackleg lesion per leaf were sampled to reach 200-250 isolates. Isolate characterization was done by classical cotyledon tests, by molecular PCR, or by HRM PCR technique which was improved in recent years.

Results: The HRM PCR distinguished different alleles of the *Avrlm4-Avrlm7* locus and is a very powerful and time saving method. We found avirulent isolates with *Avrlm7* present on *Rlm7* hybrids, which confirmed previous results. This interaction produced foliar lesions but no further development of the disease was observed. There was an increase of virulent *Avrlm7* sub-population on *Rlm7* hybrids and on non-*Rlm7* hybrids. It would appear that the specific resistance *Rlm7* seems to be broken down. All virulent *Avrlm7* isolates are also found to possess the avirulent allele *Avrlm3*. These interactions are under further investigations at INRA Bioger.

Conclusion: Break down may occur after seven years of intensive use of *Rlm7* genotypes. Nevertheless agronomically this disease dynamic is under control and final symptoms are still very low (G2 index). Several arguments may explain such situation. It may be due to the high frequency of dry autumns in the region, or due to a fitness deficit of *Avrlm7* isolates, or due to interactions with other resistance genes (QTLs or Rlm3).

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Transfer of sclerotinia resistance from wild relative of *Brassica oleracea* into *Brassica napus* using a hexaploidy step

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Background: Stem rot caused by fungal pathogen *Sclerotinia sclerotiorum* is a great threat for rapeseed (*Brassica napus*) production around the world. A high level of resistance against *Sclerotinia sclerotiorum* was documented in wild *B. oleracea* (*B. incana*), but not in cultivated rapeseed (*B. napus*) (Mei et al. 2011). Inheritance investigation and QTL analysis revealed the additive genetics for *Sclerotinia* resistance in *B. incana* (Mei et al. 2013).

Objectives: This study was conducted to transfer *sclerotinia* resistance from *B. incana* into rapeseed.

Methods: A strategy was proposed using hexaploids (AACCCC) derived from crosses between the wild *B. oleracea*-related *B. incana* genotype 'C01' and the Chinese rapeseed variety 'Zhongshuang 9' as a bridge. Progenies (BC1F1 and BC1F2) that generated by backcrossing the hexaploid to 'Zhongshuang 9' were screened firstly by five molecular markers linked to the major resistance QTL in *B. incana* and secondly by resistance evaluation. Resistant individuals in BC1F2 were checked for chromosome numbers.

Results: Among 73 BC1F1 individuals, eleven that harbouring resistance loci were selected to develop vegetative clones for resistance evaluation. Of these, five exhibited significantly higher resistance than 'Zhongshuang 9' and the most resistant individual was chosen to develop the BC1F2 progeny. Finally, five individual genotypes with nearly two-fold higher resistance than 'Zhongshuang 9' were identified from 100 BC1F2 individuals. Hereof, one rapeseed-type individual with 38 chromosomes and good self-fertility (15.0 ± 3.56 seeds/pod) was identified.

Conclusions: Our results indicate that the proposed strategy is effective for transferring sclerotinia resistance from *B. oleracea* into rapeseed.

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375
POSTERS THEME B

Investigating the durability of blackleg resistance genes in *B. napus* and the emergence of virulent isolates in *L. maculans*

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Background: *Brassica napus* (Canola/oilseed rape) is grown extensively in Australia, North America, Europe and Asia. This crop is susceptible to a few major diseases including blackleg or stem canker caused by the ascomycete fungus *Leptosphaeria maculans*. The disease has a great economic impact worldwide due to serious yield loss. A number of single dominant R-genes to *L. maculans* have been identified in different *Brassica* species; however a single R-gene may not offer durable resistance to the disease. Studies have shown the breakdown of single resistance gene, LepR3 and Rlm1 in Australia and France, respectively. In recent years Rlm3 has been reported to have broken down in Canadian canola fields. This indicates the need for proper management strategies for the development of host resistance. Moreover, effective management of resistance depends on the understanding host-pathogen interactions as well as pathogen adaptation.

Objectives: The aim of this study is to generate useful data to implement a canola cultivar rotation strategy in the Canadian Prairies based on an understanding of R-gene durability and the nature of emergence of virulent blackleg isolates.

Methods: The experimental design of the project consisted of 10 separated plots with 3 replications; 5 plots for examining durability of a single R-gene and 5 plots for determining the virulence changes in isolates. Each plot was sprayed with either 90% avirulence isolates for durability or 100% avirulence isolates for emergence. Five single Rlm Topas lines developed by AAFC Saskatchewan were used in the study along with a Topas check as a control (no R-genes). Each plot was set up for canola-wheat-canola rotation over 2-years. In addition, the Topas lines carrying different single R-genes were rearranged randomly for both trials. Disease incidence, disease severity and representative yield were recorded from all plots. Moreover, the pathogen isolates collected from either infected stubble or a 7-day Burkard spore trap were subjected to differential testing as well as PCR identification for the presence of six avirulence genes to monitor changes in pathogen virulence.

Results: Disease incidence varied among the cultivars in both the durability and emergence trials; however, similar level of disease incidence was observed in each cultivar between the trials. The emergence trial revealed higher disease severity of 2.5 on a 0-5 scale in the cultivar harbouring the single R-gene, Rlm3. The rest of the cultivars showed ≤ 1.0 disease severity irrespective of trial. PCR analysis of *Leptosphaeria maculans* isolates cultured from the stubble indicated that the isolates were carrying AvrLm5, AvrLm6 and AvrLm11. PCR analysis of ascospores collected via the spore trap indicated the presence of *Leptosphaeria maculans* as well.

Conclusions: The first year of data collection indicates the possibility of Rlm3 break down and adaptation of the pathogen. The study will continue for four years to observe the likelihood and timeline for the breakdown of R-genes and the emergence of new races. The data will be informative in planning canola rotation on the Canadian prairies.

Role of non-conventional chemicals in induced resistance against *Sclerotinia sclerotiorum* the incitant of Sclerotinia stem rot of Indian Mustard

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Background: Sclerotinia stem rot of Indian mustard incited by *Sclerotinia sclerotiorum* has become important disease in recent times in India. Wide host range, soil borne nature, difficult to manage with fungicides and lack of complete genetic resistance in all economical crops prompted us to study on the induction of systemic resistance against this pathogen. However, it is well known that plants in many crops have evolved an array of defense mechanism to combat invasions by plant pathogens. But, in crops where this mechanism is lacking, the induced resistance can be activated by treatment of plants with either natural or systemic chemicals (Kessmann et al., 1994).

Objectives: This fungus produces oxalic acid, which creates an optimal pH for the activity of enzymes related to pathogenesis. The non-conventional chemicals when apply can induce some defense through their activities on biochemical changes in plants. Hence, the present study was under taken to screen non-conventional chemicals and to examine the changes due to these in biochemical parameters.

Methods: Forty-five to fifty days old plants raised in the pots in screen house were sprayed by non-chemicals and after 24 h pure culture of pathogen was inoculated by stem inoculation method and observations on disease severity was calculated using standard scale. In control, the plants were sprayed with sterile water before challenge inoculation. Five plants/pots were maintained and the pots were arranged in CRD with four replications. Leaf tissues were taken at various intervals (0, 3 and 6 days) after pathogen inoculation from both control and treated plants and immediately homogenized and stored in liquid nitrogen. These samples were powdered and used for analysis of different biochemical changes that have taken place in the host tissues.

Results: As the concentration of different non conventional chemicals increased from 10, 50 and 100 ppm there was reduction in Sclerotinia rot disease. Maximum induction of resistance was recorded by the application of salicylic acid at 100 ppm concentration after six weeks of challenge inoculation with *S. sclerotiorum* followed by acetyl salicylic acid, indole butyric acid and indole acetic acid. Total phenols, PPO, PAL, PO and chitinase activity was at peak after 3 days of challenge inoculation with *S. sclerotiorum* and there after the activity of all these parameters slightly declined after 6 days of inoculation in all non-conventional chemicals tested. However, SA has shown maximum rise of total phenol at 3 days after challenge inoculation followed by ASA and IBA, respectively. Similarly, PPO, PAL, PO and chitinase contents were increased to the maximum in treatment with SA followed by ASA and IBA.

Conclusion: The application of SA at 100 ppm concentration significantly enhances the activity of total phenol, PPO, PAL PO and chitinase after challenged inoculation of the pathogen and activates systemic resistance against stem rot disease in Indian mustard.

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377
POSTERS THEME B

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Pseudomonas chlororaphis PA23 biocontrol of *Sclerotinia sclerotiorum* on canola: Understanding populations and enhancing inoculation

Background: Biological control is emerging as an attractive tool to manage plant diseases due to an increasing demand for sustainable approaches with minimal adverse environmental impact. Biocontrol by *Pseudomonas chlororaphis* strain PA23 has previously been shown to effectively control *Sclerotinia sclerotiorum*, one of the most important diseases of canola in Western Canada (Fernando et al, 2007). PA23 biocontrol is mediated through the production of secondary metabolites that inhibit fungal growth. These compounds include the antibiotics pyrrolnitrin and phenazine, along with HCN, protease and lipase (Poritsanos et al, 2006).

Objectives: The purpose of this research is two-fold. Firstly, to determine how time of application affects PA23 biocontrol. This will reveal at what point post inoculation PA23 begins to produce antifungal metabolites; how long PA23 populations survive on canola; and the period of time PA23 affords canola protection from Sclerotinia infection. Secondly, to research the effects of supplements on PA23 population size and antifungal activity. These findings will be valuable for designing inoculation formulations that promote PA23 establishment and biocontrol in the environment.

Methods: Antifungal metabolite gene expression will be monitored in vitro using quantitative reverse transcription PCR (qRT-PCR) at different bacterial growth phases (log and stationary growth). In vivo, we will investigate how long PA23 provides control by inoculating PA23 at different times (1, 4, 7 days) prior to *Sclerotinia ascospore* application. Petal infestation and plant infection by the fungus will be assessed. PA23 populations on petals will also be monitored by washing the petals and plating for colony forming units (CFU). The impact of supplements on antifungal activity will be assessed in radial diffusion assays, after which qRT-PCR will be used to determine how be tested for control of Sclerotinia in a greenhouse setting.

Results: Preliminary results suggest that PA23 provides long term control of Sclerotinia in a greenhouse setting. When PA23 was inoculated 7 days prior to ascospore inoculation, there was greater control than either 4-day or 1-day advance inoculation. qRT-PCR suggests that the antibiotic pyrrolnitrin is upregulated in stationary phase. Initial radial growth assays indicate that glucose and fructose amendment provides PA23 with better conditions to retard Sclerotinia mycelial growth.

Conclusions: This research will allow for a better understanding of how *P. chlororaphis* strain PA23 interacts with canola and imparts biocontrol over time. Understanding PA23 population dynamics, antifungal gene expression, as well as optimal application formulations is necessary before this bacterium is ready for implementation into a sustainable disease management strategy.

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Studies of clubroot (*Plasmodiophora brassicae Wor.*) on oilseed rape in the Czech Republic

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Background: The clubroot, caused by *Plasmodiophora brassicae*, previously a problem in vegetable, is appearing on oilseed rape in the Czech Republic. The infested stands are reported across the whole country, mainly in north and north-east of the country. The pathogen is probably spread in the whole country; its occurrence depends on weather conditions during the sawing period (in mid-August). Research on *P. brassicae* in the Czech Republic is therefore important for the development of effective management strategies under Czech environmental conditions.

Objectives: The prevention is only way of protection against clubroot. When the field is already infested solution could be using resistant winter oilseed rape cultivars, but the right crop rotation is essential too. Therefore, one part of research is targeted on resistant cultivars. Pathotype monitoring is necessary for complex knowledge of clubroot. Insight on the amount of inoculum – the spore load, which can be done by quantitative PCR analysis (qPCR) is valuable for assessment of pathogen potential.

Methods: Experiments with clubroot resistant cultivars were made in the field and greenhouse. In the greenhouse (conducted in 2013, repeated in 2014), six clubroot resistant cultivars were grown in infested soil collected from 14 Czech fields, and assessed for disease severity. The presence and amount of clubroot inoculum in soil samples was also tested by conventional and qPCR analysis. In the field, seven resistant cultivars were grown on clubroot infested field, disease development monitored monthly. Yields were measured during cropping. The experiment will be repeated in season 2014/2015. Finally, a set of 16 *P. brassicae* field isolates from across the Czech Republic were assessed for pathotype designation on the differential hosts of Williams, Somé et al., and the European Clubroot Differential set.

Results: Greenhouse testing brought various results, depending on source of soil samples. The control cultivar was highly infested with one exception. All cultivars showed good resistance on all tested localities. Index of Disease (ID) was lower than 25 % (except one). The results from both the field and the greenhouse are similar. All resistant cultivars showed good level of resistance on infested field (except one). The control cultivars were highly infested. The pathotype designation revealed 6 pathotypes according Williams classification (the most frequent pathotype 7). Somé et al. classification identified 3 pathotypes (the most frequent P3). ECD set determined 8 pathotypes (the most frequent ECD 16/14/15). The qPCR analysis revealed high spore load in infested soil samples, highest in locality Modlibohov (5 mil. spores/1 g soil) and the lowest in locality Zirovnice (70000 spores/1 g soil).

Conclusions: Tested clubroot resistant cultivars are suitable for growing in clubroot infested soil and can be recommended for agricultural production as one of the protection ways. The PCR and qPCR are reliable methods for clubroot detecting and quantification. The identified pathotypes can be useful in testing of new clubroot resistant cultivars.

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379
POSTERS THEME B

COLEOTOOL: Development of molecular tools to identify the main weevil species attacking oilseed rape, and their parasitoids

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Background: In Europe, the oilseed rape crop is very dependent on insecticides. The first way of reducing pesticide use is to guarantee the quality of diagnosis in the fields to avoid unnecessary treatments. For instance, it is very difficult to identify weevil larvae. Another way is to develop new crop protection strategies, for example by enhancing biological control in agroecosystems. Today, references and knowledge about natural enemies are poor. In France, the last inventory of the parasitoids of oilseed rape pests date back to the 60's. It is in this context that the French Ministry for Agriculture is supporting this 3 years project that has started in 2014.

Objectives:

1) To establish an inventory of parasitoid species specific to the key weevil pests of oilseed rape.

2) To provide validated molecular tools to identify weevils at any stages, and their parasitoids.

Methods: Weevils and parasitoids are caught by beating, Malaise traps or yellow traps in several fields all around France. Weevil larvae or eggs are reared in a laboratory to identify the hosts of each parasitoid species. The COI mitochondrial fragment and the ITS2 nuclear marker will be sequenced. Theses sequences will feed a database that will be searchable by BLAST. The molecular identification tool will be validated and tested in field experiments in the last year of the project.

Results:

1) To give a clear picture of weevils' parasitoids in France. Revisions of the Zoological Nomenclature could be done if necessary.

2) To develop validated and operational methods to identify at least 40 insect species at any stage of development thanks to sequences.

Conclusions: The main goal of this project is to develop molecular tools that will be available online. These tools should make it possible to identify and hopefully quantify insects (pests and natural enemies) in different kind of environments and at different stages. This should lead the way to new researches to identify agricultural practices or landscape impacts on the pests and their natural enemies.

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Identification of races of *Leptosphaeria maculans* infecting high erucic acid rapeseed (HEAR) in western Canada

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Background: Erucic acid is a very important raw material for the industry and can be used as an additive in to lubricants and solvents, a softener for textiles and an amide derivative in polymer synthesis. HEAR contains 43% minimum erucic acid in the seed oil and is the most economical source of this long-chain fatty acid (Mastebroek HD, et al. 1997). HEAR is grown by contract growers. Blackleg disease is caused by the pathogen *Leptosphaeria maculans* (Desmaz.) Ces. &De Not., and is responsible for significant yield loss in oilseed rape and canola (*Brassica napus L.*) worldwide. Genetic resistance has been used effectively to control blackleg in western Canada. Specific resistance genes are an effective means of disease control when the pathogen population is mainly avirulent on the cultivated varieties carrying the corresponding resistance gene (Kutcher, H.R. et al, 2010). However, the resistance has been breaking down in recent years due to changes in the pathogen populations and new races.

Objectives: The objective of this study is to determine the race structure of *L. maculans* from stubble samples collected in HEAR fields in western Canada. The pathogen structure information could be used in the development of blackleg disease control strategies and breeding for resistant cultivars of canola and rapeseed varieties of HEAR in western Canada. This research will provide a current picture on the distribution of different races and potentially detect novel races.

Methods: Blackleg infected stubble was collected from the HEAR commercial fields in western Canada in 2014 and 2015 in Manitoba, Saskatchewan and Alberta. The pathogen was isolated from infected stubble onto V8 juice media. Single spore isolates were then cultured from pycnidia. Multiplex PCR was used to discriminate between *L. maculans* and *L. biglobosa*. PCR was performed on *L. maculans* isolates using primers obtained from *AvrLm1, AvrLm5, AvrLm6, AvrLm4-7* and *AvrLm11*. For Avr genes which have not been cloned were characterized using a differential set of *B. napus* genotypes. Inoculations were made using single-spore cultures, diluted to a 2 x 107 CFU/ml and inoculated onto wounded cotyledons seven days after planting. Phenotypic evaluation was made 10 days after inoculation using a 0-9 scale (Sawatsky, W. M, 1989).

Results: Preliminary results indicate the frequency of avirulence alleles *AvrLm1, AvrLm 2, AvrLm3, AvrLm4, AvrLm6, AvrLm7, AvrLm9, AvrLmLepR1 AvrLmLepR2* and *AvrLmLepR3* varied from 5 to 100% depending upon the geographic location. Four avirulence alleles were observed at a higher frequency, including *AvrLm2, AvrLm4, AvrLm6* and *AvrLm7* (77 to 100%). The corresponding R genes (*Rlm2, Rlm4, Rlm6 and Rlm7*) could be candidates for use in canola/rapeseed cultivar development in western Canada.

Conclusions: This knowledge, coupled with the knowledge of the existing R genes present in current canola/rapeseed cultivars could be used to aid in cultivar development. This information could also help canola/rapeseed growers in the selection of the appropriate cultivars for different growing regions in western Canada. This updated race structure data for *L. maculans* in western Canada is helpful in the development of improved blackleg disease resistance strategies that might prevent or delay resistance breakdown. This research could improve the management of blackleg using cultural methods, by reinforcing the importance of crop rotation to producers and the industry.

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381
POSTERS THEME B

Variation for pod shatter resistance in an international germplasm collection of *Brassica rapa*

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Background: Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* is a major factor limiting the production potential of *Brassica* oilseeds. Synthetic chemical fungicides used for its management do not provide satisfactory control. At present, there is no reliable resistance source available in cultivated *Brassica*. Three sets of *B. juncea* introgression lines from already reported resistant wild, E*rucastrum cardaminoides* and *E. abyssinicum* (Garg et al. 2010) were used in these studies.

Objectives: Stabilizing and enhancing resistance responses of introgression lines to diverse *S. sclerotiorum* isolates.

Methods: About 7,000 plants from three introgression sets were screened against *S. sclerotiorum* isolate (Ludhiana), using stem inoculation technique described by Buchwaldt et al. (2005). These were narrowed down to 617 and then 94 progenies over three selection cycles. Introgression lines showing consistent reaction over three years formed the basic germplasm to initiate next phase of phenotypic selection to further improve average resistance responses. In the second phase, selected progenies were challenged by four diverse *S. sclerotiorum* isolates (Bharatpur1, Bharatpur2, Ludhiana and Bawal). These four isolates were separated in to different clades on the basis of morpho-cultural and molecular variation. Average lesion length and proportion of plants showing hypersensitive response were used as selection criteria.

Results: After three years of rigorous screening; 22, 42, 66, 71 and 36 progenies were selected based on their consistent resistance responses to all four isolates. Intensive selection led to a significant decline in mean lesion length. Against Bharatpur-1 isolate, the lesion length declined from 4.41 cm to 3.65 cm while, frequency of plants showing hypersensitive reaction (HR) remained almost constant. Similar trends were also observed against Bharatpur 2, Ludhiana and Bawal isolates. These sets showed decline in average lesion length from 5.38 to 2.69, 4.52 to 3.67 and 7.09 cm to 3.53 cm, respectively. Twenty two progenies were identified for resistance against all the four isolates. Of these, four progenies namely, ARL-42, A-825-1, JBR-31 and ADRL-7 exhibited consistent resistant reaction over the six years. These progenies showed lowest mean lesion length for all the four isolates. These progenies bred true for their resistant responses and also showed minimal within-progeny variation.

Conclusion: Six cycles of selection enhanced and stabilized resistance against *S. sclerotiorum*. Four progenies exhibited consistent resistant responses over six years of selection. All the selected progenies carry euploid chromosome number and bred true for resistance. Molecular characterization is underway.

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382
POSTERS THEME B

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Characterization of *Sclerotinia sclerotiorum* necrosis inducing proteins

Background: *Sclerotinia sclerotiorum* causes stem rot in *Brassica napus* which leads to lodging and severe yield losses. The fungus releases acids, enzymes and toxins that destroy the host before it can mount a defense. As it consumes the necrotic tissue, the rot spreads leading to stem collapse. *S. sclerotiorum* secretes two necrosis and ethylene-inducing proteins (SsNEP1 and SsNEP2) that cause significant necrosis when infiltrated into plant tissues (Bashi et al. 2010). Necrosis is also caused by polygalacturonase enzymes (SsPG3 and SsPG6) which can be partially overcome by the expression of *B. napus* polygalacturonase inhibitor proteins (Bashi et al., 2013).

Objectives: The objectives of this study are: (i) to use transcriptomic and bioinformatic analyses to identify additional candidate necrosis-inducing effectors (NEs) from *S. sclerotiorum*, and (ii) to examine their contribution to disease using an in planta expression system and *S. sclerotiorum* gene knockouts. Ultimately, these NEs may be used to develop an effector-assisting breeding strategy to screen *B. napus* cultivars for stem rot resistance.

Methods: The genomic sequence of *S. sclerotiorum* isolate 1980 has been published. The proteins encoded by these genes were subjected to several bioinformatic filters to identify small, secreted proteins. Illumina RNA-seq analysis was conducted to obtain information about the expression of all *S. sclerotiorum* genes through the earliest (1 hours) to the latest (48 hours) stages of infection on *B. napus*. Proteins were considered to be candidate NEs if they passed the bioinformatic filters and if the expression of their corresponding genes was induced within 1-6 hours of inoculation as these proteins are most likely to impact host-pathogen interactions leading to pathogen establishment. The candidate NEs were tested for their ability to cause necrosis using an *Agrobacterium tumefaciens* transient infiltration system.

Results: Potential *S. sclerotiorum* NEs were identified using computational and RNA-seq analyses. The *S. sclerotiorum* genome encodes ca. 900 secreted proteins (i.e. possess a signal peptide), of which 100 were found to be small (less than 250 amino acids), cysteine-rich proteins that do not possess transmembrane domains, a GPI-anchoring site or a vacuolar sorting (NPIR) motif; these are signatures of secreted NE proteins. The expression of these candidate genes was determined from the RNA-seq information, yielding 26 that were induced to relatively high levels during the early phases of the infection. These were subjected to in planta expression and several new *S. sclerotiorum* NEs were identified.

Conclusions: *S. sclerotiorum* secretes a substantial repertoire of proteins that can induce necrosis in host tissue and contribute to disease progression and severity in *B. napus*. These may serve as targets for developing resistance to stem rot disease.

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383
POSTERS THEME B

Genetic diversity among isolates of *Albugo candida* in India using newly identified SSR markers

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Background: White rust caused by an obligate oomycete *Albugo candida* affects both the vegetative and reproductive stages of the crop. Variability among the *A. candida* isolates has not been studied adequately in India. Knowledge of population structure of a plant pathogen at genetic level is expected to provide greater insights for understanding the relation between disease severity and prevalence of particular genetic group (s) of fungus. Simple Sequence Repeat (SSR) markers are widely accepted for studying genetic variability within a fungal species due to high reproducibility, multi-allelic nature, relative abundance, wide range of distribution and high mutational rate (Schlotterer 2004).

Objectives: Development and validation of new SSR markers for *A. candida* and their use for establishing genetic variability among *A. candida* isolates in India.

Methods: The availability of *A. candida* whole genome sequence (Links et al. 2011) provided a chance to identify SSRs in silico. *A. candida* genome is represented by 252 scaffolds covering 34.5Mbps of the genome. This genome sequence was used for in silico identification of SSR motifs using MISA perl script. White rust infected leaf samples of B. juncea were collected from mustard growing areas of north-west India. A set of twenty new designed SSR markers were used to establish genetic diversity among eleven *A. candida* isolates.

Results: In total 145 SSRs were identified from the genome sequence of *Albugo candida*. Twenty primer pairs were synthesized. These markers proved to be pathogen specific as there was no amplification when DNA from uninfected mustard leaves was used as template. Eighteen polymorphic markers amplified a total of 54 alleles with an average of 3 alleles per locus. Primer pair for AcSSR1 showed the maximum PIC value (0.769). Eleven isolates could be resolved into four clusters. The first cluster comprised of isolates AcMs, AcFr and AcBt. The second cluster included four isolates (AcJs, AcMk, AcKg and AcDh), the fourth cluster consisted of three isolates (AcRp, AcLg and AcLd2b) while third cluster included only one isolate from foot hills of Himalaya (AcHr).

Conclusion: Genome sequence is needed to design molecular markers such as ITS, COS2 and LSU, and could be applied to establish the interspecies differences rather intraspecies variations. Newly designed SSR markers were effective in revealing the genetic diversity among the different isolates of *A. candida* and could help unravel the high degree of genetic diversity among the *Albugo* species complexes.

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Identification of pathogenicity of *Plasmodiophora brassicae* from three different regions in Sichuan, China

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Background: Clubroot disease in *Brassicaceae* is caused by a soil-borne pathogen, *Plasmodiophora brassicae*. Different physiological races or pathotypes in *P. brassicae* have been identified in China, among which the race No.4 was considered the prevailing race in the Sichuan plain area (Shen et al. 2009; Ding et al. 2013; Ji et al. 2013). Pathogenicity of different races may be largely different to host plants. Therefore, identification of physiological races or pathogenicity of pathogens from different rapeseed (*Brassica napus L.*) regions is an important fundamental task for control of clubroot in rapeseed production.

Objectives: To mainly observe the difference in pathogenicity of *P. brassicae* from three different severely infested areas in Sichuan Province, China.

Materials and methods: In present study 20 rapeseed materials were used, including 19 inbred lines and 1 hybrid variety. "Deyou 6", the present control variety of regionalized tests of rapeseed in Sichuan Province. Clubroot tumors and infested soils were collected from three different regions in Sichuan Province, including Guanghan (plain area), Dayi (plain area) and Kangding (western high-mountain area), for the tests. Two methods were used to identify the difference in pathogenicities of the pathogens: 1) artificial inoculation with spores isolated from the root tumors, and 2) direct test with infested soils. Artificial inoculation was conducted by injection of 1ml spore suspension with a density of 1 x 107 spores / ml into soil near the seedlings at 2-leaf stage. Tests were made in 4 replications. The disease occurrence was observed with 20 plants for each line 50 days after inoculation or seeding. Disease incidence (DI) and severity index (SI) were calculated based on the disease observations.

Results: In the artificial inoculation test, average DI's were 63.36%, 32.83%, 4.29%, respectively; average SI's were 39.1, 1.71, 2.1, respectively, for the 3 different source of pathotypes. In the soil test, average DI's were 64.49%, 29.08%, 12.73%, respectively; average SI's were 46.96, 18.81, 8.97, respectively. It was obvious that the Guanghan pathotype showed the strongest pathogenicity and the Kangding pathotype showed the weakest pathogenicity. Results of the two methods were consistent.

Conclusions: The pathogenicities of *P. brassicae* from the three different regions in Sichuan Province were different. The orders of occurrence were Guanghan pathotype > Dayi pathotype > Kangding pathotype.

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385
POSTERS THEME B

Report on a new insect pest on oilseed rape in Tibet, China

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Background: Oilseed rape is a very important oil crop in China. It can be damaged by many insect pest species and many yields were lost in every year. More and more new insect pest species begin to damage oilseed rape because of the climate and crop cultivation system change now. Investigate and study new insect pest species that damage on oilseed rape are very important and significant to control them.

Objectives: A new insect pest on oilseed rape was found in Tibet, China, in 2014. Its bio-characteristics, occurrences rules, harmful characteristics and what species it is should be studied by systemic investigation and filed census. Then we can make a safe and high-efficiency standard procedure to control them.

Methods: The new insect pest bio-characteristics were observed by stereomicroscope, occurrences rules and harmful characteristics were investigated by filed systemic census. According to the bio-characteristics, occurrences rules and harmful characteristics compare them with literature to identify what species it is. Using agricultural, physical, biological and chemical methods to integrate control them.

Results: The insect pest adults appear in spring, first appear in April later to early May. They damage oilseed rape seedlings from cotyledon to three leaves stage. The adults eat leaves. Some adults also can be found under ground near to the roots of oilseed rape. There are more than 30 adults per square meter in filed when we investigated in June 17, 2014.

The size of adults are 6.1~7.9mm long, the pronotums are 1.7~2.2mm wide and the backs are 2.3~3.0mm wide. They have two geiculate antennae, each antenna has 9 segments. The scapes are 0.7~0.9mm long and the flagellums are 1.0~1.4mm long. The ends of antennae are a little more swollen. All adults are black color, prognathous mouthparts. They have some microgrooves on its two elytrons, but they can not fly.

Conclusions: The new insect pest belongs to *Leptomias* sp., *Culculionidae, Coleoptera*. It has not been identified and confirmed what species it is yet.

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Deciphering the role of cyclophilin a (Cyp4) in blackleg causing fungi *Leptosphaeria maculans* and *L. biglobosa* on oilseed rape

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Background: Oilseed rape is challenged by a number of fungal pathogens. One of the most economical important diseases is phoma stem canker (blackleg) that cause serious loses globally. The disease is caused by the fungal species complex *Leptosphaeria maculans* and *L. biglobosa*. However, several studies have shown that *L. maculans* is much more aggressive on oilseed rape (Shoemaker and Brun, 2001). This phenomenon can only in small parts be explained by its attribute to produce the host-unspecific toxins sirodesmins (Sock and Hoppe, 1999), which are not produced by *L. biglobosa*. However, it is still unclear which main factors underlie these huge differences of aggressiveness. In spite of the fact that cyclophilins are highly conserved family throughout genera, however the gene is not well studied in *L. maculans* and *L. biglobosa* (Singh et al., 2014). Similarly, it has been shown that cyclophilins may contribute to the virulence of certain fungal pathogens (Viaud et al., 2002).

Objectives: To perform comparative analysis of cyclophilin gene family in *L. maculans* and *L. biglobosa*. Functional characterization and delineating the precise role of cyclophilin A (Cyp4) in virulence.

Methods: Genome-wide identification of cyclophilin gene family, Culturing of various fungal isolates, Pigmentation and sirodesmin analyses, Host pathogen interactions, RNA isolation and cDNA synthesis, qRT-PCR and cloning of Cyp4.

Results: Through comprehensive whole genome analyses of *L. maculans* and *L. biglobosa*, we identified seventeen and fifteen genes respectively encoding cyclophilin in these two fungi. Further to gain more insight, in silico analyses of sequences followed by cloning of cyclophilin A (Cyp4) in various isolates showed the compelling differences between two species at sequence level as well. In addition, expression levels of the Cyp4 in the mycelium found to be relatively high in *L. maculans* as compared to *L. biglobosa.* However, the expression analyses not only demonstrated a significant difference among species but also significant intraspecific variation. Furthermore, pigmentation and sirodesmin analyses distinguished *L. maculans* and *L. biglobosa* isolates. Disease assessment studies of *L. maculans* and *L. biglobosa* isolate on various oilseed cultivars also exhibited different level of pathogenicity.

Conclusions: Taken together our finding for the first time shed light onto the significant differences between the two species at sequence level. In addition ongoing ad planta studies may further support our hypothesis that cyclophilins and their expression may explain the difference in virulence on oilseed rape against *L. maculans* and *L. biglobosa.*

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387
POSTERS THEME B

Shotgun label-free proteomic analysis of clubroot resistance mediated by the resistance gene *Rcr1* from *Brassica rapa* ssp. *chinensis*

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Background: Clubroot disease, caused by the soil-borne phytopathogen *Plasmodiophora brassicae* Woronin, is one of the most serious diseases on *Brassica* crop species worldwide. For decades, host resistance has been the major tool in management of clubroot. Although several resistance genes were characterized and employed in commercial cultivars, mechanisms of clubroot resistance, especially the molecular mode of action, remains sketchy.

Objectives: The major purpose of this work was to profile and compare the proteomes of canola plants with and without the clubroot resistance (CR) gene *Rcr1*. The functional annotation of differentially accumulated proteins (DAPs) identified by the proteome comparison was analyzed to reveal their biological context. The biological pathways associated with DAPs were highlighted and subjected to biochemical and genetic studies.

Methods: A segregating BC1 *B. rapa* canola population was inoculated with *P. brassicae* and genotyped using flanking markers for the presence/absence of Rcr1 gene. The total proteins were extracted from root tissues at 2 weeks after pathogen inoculation and analyzed using UHPLC-MS/MS. The spectra collected from the MS were processed through the global proteome machine (GPM) software using the X!Tandem algorithm. The abundance of each identified protein was calculated as normalized spectral abundance factor (NSFA), which was then subjected to a t test to determine DAPs. The identified DAPs were annotated using Blast2GO based on gene ontology terms and their expression patterns were analyzed using the Mapman software.

Results: A total of 527 DAPs were identified in samples carrying *Rcr1*, relative to those without this CR gene. Functional analysis of DAPs identified a potential signaling pathway associated to the resistance by *Rcr1* that was distinct from other commonly reported modes of recognition receptors for fungal and bacterial pathogens. This novel signaling pathway appeared to act in a calcium-independent way through an unknown cascade of mitogen-activated protein kinases (MAPK) and would require the ubiquitin-26S proteasome, which was previously demonstrated to function in abiotic stresses, especially the cold stress. Furthermore, our study also identified a range of biological processes that were differentially regulated in resistant plants. The putative pathogen-induced defense responses included a potential ROS accumulation, sulfur-containing glucosinolate breakdown and lignin biosynthesis. A variety of host metabolites typically induced by *P. brassicae* in compatible interaction, including glycolysis and arginine catabolism, were significantly down-regulated in resistant plants carrying *Rcr1*.

Conclusion: The proteomic study showed that clubroot resistance mediated by *Rcr1* would consist of induced pathogen-recognition and signaling pathways in defense responses, as well as suppression of pathogen-induced re-programming of host metabolism favoring clubroot development. The results provided important insights into the mechanisms of clubroot resistance, and offered candidate targets for further biochemical and genetic studies to verify the action mode for *Rcr1* and other CR genes.

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Characterization of differentially regulated host metabolism conferred by the clubroot resistance gene *Rcr1* using metabolome profiling and targeted metabolite analysis

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Background: Clubroot disease, caused by the soil-borne phytopathogen *Plasmodiophora brassicae* is one of the most devastating diseases on *Brassica* crop species worldwide. Host resistance has proven to be the most effective and economical approach for clubroot disease control. Better understanding of clubroot resistance (CR) mechanisms, especially relating to specific CR genes, will facilitate selection and use of these genes in breeding for reliable and durable resistance. The CR gene *Rcr1* was previously identified from *Brassica rapa* ssp. chinensis, and initial transcriptomic and proteomic analyses indicated potential changes in metabolism in plants carrying *Rcr1* gene.

Objectives: The major purpose of this work was to identify and characterize the metabolic changes in a segregating *B. rapa* canola population carrying *Rcr1* gene induced by *P. brassicae*. A focus was placed on the metabolism highlighted by in transcriptomic and proteomic analyses.

Methods: Root samples were collected at 15 days post inoculation, when the secondary infection by *P. brassicae* just started but the clubbing symptoms were not yet visible. The presence of *Rcr1* was verified with flanking markers to separate plants into resistant and susceptible groups. After pulverization in liquid nitrogen, samples were extracted and analyzed study the fluctuations at the metabolome level performing liquid chromatography mass spectrometry (LC/MS) analysis using on an LTQ-Orbitrap Classic mass spectrometer. The metabolites were characterized based on search against an in-house built library for canola and quantified in comparison to the control (without *Rcr1*). In addition, synchrotron-based Fourier infrared spectroscopy was employed to profile the metabolites in the same set of sample.

Results: The global profiling of metabolites identified 83 differentially accumulated metabolites in resistant samples relative to susceptible samples. Caulilexin C, a phytoalexin derived from indole-containing compound metabolism, was significantly induced in resistant samples, along with several other indole-containing metabolites which appeared to be related to differentially regulated auxin metabolism. The defense-related phytohormone jasmonic acid, as well as several additional metabolites involved in jasmonic-acid signaling pathways, was also highly induced in pathogen-infected resistant samples. Furthermore, spectroscopic analysis revealed potential changes in plant cell-wall compound organization, particularly the lignin composition.

Conclusion: Metabolomic analysis identified putative contributions of several metabolic processes to clubroot resistance mediated by *Rcr1*. The induction of jasmonic acid as well as the intermediate molecules involved in signaling pathways indicated its pivotal role in inducing further downstream defense responses, one of which is the high accumulation of phytoalexins derived from the metabolism of indole-containing compound. The altered metabolism in the plant cell wall indicates a potential role of host-cell reinforcement against the infection into cortical tissues.

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389
POSTERS THEME B

Swede midge distribution across prairie Canada

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Background: Swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae), was first identified in North America from southern Ontario cole crops in the year 2000 (Hallett and Heal 2000). The pest and its impact spread quickly from this region to adjoining jurisdictions. Economic injury to spring oilseed rape (canola) in northern Ontario has become so severe that the current recommendation is to cease canola production in the area for 3 years. "Jumping larvae" were collected from canola in Saskatchewan in 2003, with the first swede midge identification from the Canadian prairies in 2007 in northeastern Saskatchewan. However, swede midge injury to canola crops on the Prairies was not reported until 2012, with no reports of injury to cole crops in the region to date.

Objectives: Because the pest poses a major risk to the 7 million ha canola industry on the Canadian prairies, we wished to determine the current distribution and population growth of swede midge in the region to assess the potential for economic injury here.

Methods: To determine swede midge distribution, surveys of canola fields for midge injury were conducted in 2012-14 in Saskatchewan, and swede midge pheromone traps were placed in or near canola and cole crop fields across the Canadian Prairies in 2013-2014. Emergence cages were placed over canola stubble at sites in northeastern Saskatchewan in early spring 2014 and removed in August, and at new locations in the same fields at the end of July and removed in September. Presence and number of swede midge males were determined.

Results: Swede midge adults were found on pheromone traps across Saskatchewan and several locations in Manitoba in 2014, greatly extending the known range of the insect. However, at a maximum of eight males per pheromone trap per week, numbers were much lower than typically found in eastern Canada. Visual symptoms of damage to canola occurred later in 2014 than in 2013, perhaps a reflection of the very cold and wet spring in 2014. First emergence of adults from the soil in 2014 was in early July, about 4-6 weeks later than seen in eastern Canada. Two to three generations may occur on the Prairies as opposed to the more typical four in eastern Canada.

Conclusions: The rapid expansion of swede midge across Saskatchewan and Manitoba supports the premise that it could have a serious economic impact on canola production in the region. The low numbers found may be an indication that the midge is early in its establishment phase as an invasive species, or may reflect its lower reproductive potential here than in eastern Canada, or may result from a combination of both these factors.

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Understanding effector-triggered defence responses against the phoma stem canker pathogen *Leptosphaeria maculans*

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Background: Effector-triggered defence responses of oilseed rape against the phoma stem canker pathogen *Leptosphaeria maculans* are not well understood although the importance of salicylic acid and ethylene signalling has recently been suggested. Many aspects remain to be clarified, including variation in host defence responses against *L. maculans* with different effectors or effector combinations. Furthermore, the expression of receptor-like proteins (RLPs) and their encoding genes has to be solved to better understand effector-triggered defence responses against this apoplastic fungal pathogen (Stotz et al., 2014).

Objectives: To better understand effector-triggered defence responses by challenging susceptible and resistant oilseed rape genotypes containing different resistance (R) genes with *L. maculans* isolates that differ in effector gene composition. To clarify the contribution of RLPs to race-specific resistance by studying expression of LepR3 at mRNA and protein levels.

Methods: *L. maculans* isolates used differ in expression of AvrLm1 and AvrLm4. Susceptible and resistant oilseed rape cultivars with corresponding R genes were tested. Effector-triggered defence responses were analysed using quantitative RT-PCR, diaminobenzidine and nitroblue tetrazolium staining to monitor hydrogen peroxide and superoxide production, respectively. The expression of LepR3 was studied using qRT-PCR and immunoblotting with an antibody generated against the encoded RLP.

Results: The strength of the host defence response was a function of the *L. maculans* effector combination. The expression of LepR3 mRNA was low and constitutive in a susceptible cultivar. Bands of ~80 and ~140 kDa were recognised by the antibody against the LepR3 protein. The expression of this RLP appeared to decrease in response to infection by an *L. maculans* isolate with the corresponding effector AvrLm1.

Conclusions: Effectors appear to differ in their ability to suppress host defence responses. The LepR3 antibody offers a new tool to better understand the fate of RLPs during host defence responses against *L. maculans.* Subcellular localization of the LepR3 protein during the defence responses will be needed to further characterise the process of effector-triggered defence.

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391
POSTERS THEME B

Control of cabbage stem weevil in winter and spring oilseed rape

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Background: In Lithuania, like in many other countries, rape has become one of the most promising crops recently. In the intensive cropping systems, one of the most important factors limiting rape productivity is insect pest damage. In Lithuania, little is known about the harmfulness of stem pests in rape. The area of rape has rapidly increased recently, which is expected to result in the invasion from cabbage stem weevil (*Ceutorhynchus pallidactylus*) in rape fields.

Objectives: The study was aimed to estimate *C. pallidactylus* infestation level in rape and to determine the optimal application timing of insecticides with a different mode of action.

Methods: Experiments were conducted in the winter and spring rape crops in the Institute of Agriculture, LRCAF, in 2011–2013. Contact insecticide lambda–cyhalothrin 0.15 l ha-1 and systemic insecticide thiacloprid 0.3 l ha-1 were used in the experiments. *C. pallidactylus* control included the following treatments: unsprayed; cover spray against pollen beetles and pod pests with deltametrin 0.15 l ha-1; contact and systemic insecticides were applied when plants were <10 cm, 10–20 cm, 20–30 cm and insecticides were applied twice (< 10 cm and 20–30 cm). Thirty samples of rape stems per plot were taken to be examined for pest damage.

Results: The main flight of *C. pallidactylus* started at the end of stem elongation in winter rape and at plant emergence in spring rape. *C. pallidactylus* infestation in spring rape was 2.2-4.3 times less compared to winter rape. At all application times, contact and systemic insecticides effectively reduced the number of pest-injured stems in winter rape. Compared with the control and cover spray plots, a significant yield increase and the highest benefit was obtained in winter rape treatment applied with systemic insecticide at the beginning of active migration of adults, when stems were 10–20 cm high (GS 39). In spring rape, both insecticides at all application times significantly reduced the number of *C. pallidactylus* larvae-injured stems compared with control plots, and in some cases compared with cover spray plots. However, seed yield increased when systemic insecticide had been applied when spring rape was 20–30 cm high (GS 53). It is likely that systemic insecticides applied against *C. pallidactylus* at the beginning of spring rape inflorescence emergence provided control of pollen beetle (*Meligethes aeneus*) too.

Conclusions: At all application times, contact and systemic insecticides effectively reduced the number of *C. pallidactylus*-injured stems in winter and spring rape. A significant yield increase of winter rape was obtained only when systemic insecticide had been applied at the beginning of active migration of adults at 10–20 cm plant height. Spring rape productivity increased when systemic insecticide had been applied at 20–30 cm plant height and both insecticides had been applied twice.

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392
POSTERS THEME B

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Monitoring of plant and airborne inoculum of *Sclerotinia sclerotiorum* in spring oilseed rape using real-time PCR

Background: Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is a major disease of spring oilseed rape in Sweden. The pathogen survives in the soil for long periods as sclerotia. Ascospores are produced in moist conditions by carpogenic germination and constitute the major source of inoculum for infection by *S. sclerotiorum*. Infection is suggested to mainly occur when ascospore-infected petals fall and stick to the leaves, allowing the pathogen to penetrate the petiole and infect the stem. The impact of Sclerotinia stem rot is dependent on weather conditions and the timing of ascospore release, the disease causes severe damage which varies from year to year in spring oilseed rape in central Sweden, and thus oilseed rape fields are presently subject to large-scale chemical treatment. The disease forecasting service available to Swedish farmers is a regional risk assessment based on local climate and field information and is like other forecasting schemes not a satisfactory tool for individual fields.

Objectives: Our objectives were to develop and validate a *S. sclerotiorum* specific real-time PCR assay that has the potential to be used as a tool in a field specific disease risk assessment and to increase our knowledge about the infection process by determining the presence of natutrally occurring inoculums (ascospores) of *S. sclerotiorum* (i) on oilseed rape petals, (ii) on leaves of the plant and (iii) in air using collection tapes from a Burkhard spore sampler.

Methods: A real-time PCR assay was developed and used to determine the incidence of *S. sclerotiorum* DNA on petals and leaves of spring oilseed rape as well as in air samples. Five field experiments were conducted from 2008 to 2010 to detect and study pathogen development. The presence of Sclerotinia DNA on petals and leaves at different leaf levels of the plant of two different cultivars was determined regularly during the flowering period. Air samples were collected using a Burkad 7-day continuously recording spore sampler starting in late May 2010.

Results: The real-time PCR assay proved fast and sensitive and the relationship between percentage of infected petals determined using a conventional agar test and the PCR assay was linear (R2>0.76). There were significant differences in *S.sclerotiorum* incidence on petals at different stages of the flowering period. The incidence of *S.sclerotioum* DNA on the leaves varied (0-100%), with significantly higher incidence on leaves at lower leaf levels of the plant. In one field experiment, *S sclerotiorum* DNA was not detected on petals during flowering, whereas the pathogen was detected on the leaves, with a corresponiding stem rot incidence of 7 %. The amount of *S.sclerotiorum* DNA in air sampled using the spore trap revealed that the spore release did not coincide with flowering on that experimental site.

Conclusions: Using a real-time PCR assay to assess the incidence of *S.sclerotiorum* on oilseed rape leaves could potentially improve disease risk assessment in a disease support system based on predictive tests, field data and local climate.

393
POSTERS THEME B

Transferring blackleg resistance from *Brassica carinata* to canola using synthetic hexaploid species (AABBCC)

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Background: Blackleg caused by *Leptosphaeria maculans* is one of the most serious diseases in canola production particularly in Australia, Canada, and Europe (Rimmer 2006). A high level of blackleg resistance shows in *B. carinata* (BBCC) and synthetic new *Brassica* species 'Meng' with three genomes (AABBCC) from the crosses of *B. rapa* and *B. carinata*.

Objectives: Genetic resistance to *L. maculans* is considered to be the preferred approach for controlling this disease. However, the resistance determined by a single race-specific gene is not durable and has been report to be broken down. Transferring the resistance from the B genome-containing *B. carinata* and 'Meng' appears to become a feasible approach to achieve a complete blackleg resistance in canola.

Methods: The resistance to blackleg from *B. carinata* (BBCC) 'T4001' and 'Meng' (AABBCC) is transferred into the susceptible *B. napus* (AACC) 'Westar' through interspecific hybridization followed by backcrossing to 'Westar' twice or more times and selfing to produce pure lines. Blackleg isolates were used to select the resistant individuals in each generation through cotyledon inoculation. The blackleg resistance introgressed from *B. carinata* and 'Meng' into canola will be tested with various blackleg isolates belonged to PG2, PG3, and PG4. Embryo rescue tissue culture was used to obtain F1 plants of the crosses of 'Meng' and 'Westar'.

Results: When the F1 plants were backcrossed to 'Westar', the seed setting of backcrosses between 'Meng' and 'Westar' is much better than using *B. carinata* to cross with 'Westar' directly, which makes it much easier to transfer blackleg resistance from 'Meng' to canola. Currently, we observed at least two kinds of resistance interactions in the BC1S1 of 'Meng' and BC2S1 of *B. carinata* according to the inoculation results.

Conclusions: In order to further control blackleg disease, transferring new resistances from the B-genome *Brassica* species into canola has been attempted for a long time. It is not easy to obtain the complete blackleg resistance as that showed in *B. carinata* due to low fertility, poor seed set, and unstable introgression, even though the advanced breeding methods have been implemented. However, our results showed a strong possibility to transfer the high level of blackleg resistance from 'Meng' into canola.

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394
POSTERS THEME B

Analysis of the host hormonal signaling involved in *Brassica napus* - *Leptosphaeria maculans* Pathosystem

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Background: Canola (*Brassica napus* cv.) is the number one oilseed crop in Canada. Blackleg, caused by *Leptosphaeria maculans*, is the most economically important disease of canola. Evidence from previous studies has shown that plants are able to adjust their hormonal signals to combat different types of plant pathogens. Because this response is highly dependent upon the disease causing pathogen and the nature of infection within the host, it is crucial to study the hormonal signaling profile exhibited by canola when encountering *L. maculans*. Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are well known hormones involved in plant defense. The expression of genes such as WRKY70, NPR1, PR4 and BON1, have been shown to be linked to hormone signaling.

Objectives: To understand the regulation of the downstream signaling under each hormone and to determine which hormone(s) may play an important role in canola when combating blackleg, the expression of genes (e.g. WRKY70, NPR1, PR4 and BON1)controlled by SA, JA and ET signaling were monitored using RT-qPCR.

Methods: The *L. maculans* isolate HCRT75 8-1 (AvrLm2, AvrLm4-7, AvrLm6 and AvrLmS) was used in this study, as it displayed different levels of disease severities (susceptible, intermediate and resistant reactions based on lesion size on cotyledons) when inoculated onto different canola cultivars. The *L. maculans* isolate was inoculated on the cotyledons of Westar (no Rlm gene), Surpass400 (RlmS and LepR3) and 01.23.2.1 (Rlm7), and the RNA was extracted from the cotyledons at three time points (3, 7 and 10 days post inoculation). To analyze the expression levels of the marker genes under SA, JA and ET signaling, RT-qPCR was carried out on cDNA synthesized from the extracted RNA.

Results: Among the analyzed genes, some of the transcription factors controlling the defense response (such as predicted BON1) seemed to be very important for rapid and effective resistance. The expression of BON1 on 01.23.2.1 was down-regulated at 7 dpi while in Westar it was up-regulated. The onset of the expression of some genes was more important than the expression levels. For instance, the early (3 dpi) and late (10 dpi) induction of PR4 distinguished between the resistant (01.23.2.1 and Surpass400) and susceptible (Westar) cultivar. In Surpass400, however, the BON1 was induced at 7 dpi and illustrated early induction of PR4. The data from WRKY70 and NPR1 suggested that early (3 dpi) induction of SA signaling is crucial for resistance because Surpass400 and 01.23.2.1 had higher expression of these two genes than Westar at 3 dpi.

Conclusions: Results confirm that the extent of plant defense in canola against blackleg is dependent on factors involving the hormone signaling in planta. It appears that the expression levels and/or onset of these factors determined the levels of resistance.

395
POSTERS THEME B

Effect of water flooding on survival of *Leptosphaeria biglobosa 'brassicae'* in stubble of oilseed rape (*Brassica napus*) in central China

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Background: Blackleg (phoma stem canker) caused by *Leptosphaeria maculans*/*L. biglobosa* is an economically important disease on oilseed rape and many cruciferous vegetables (Fitt et al.,2008). Oilseed rape-rice rotation is a routine cultivation practice in central China.

Objectives: This study was conducted to assess the effect of flooding on survival of *Leptosphaeria biglobosa* '*brassicae*' (Lbb) in the stubble of winter oilseed rape (*Brassica napus*).

Methods: Basal stems with typical blackleg symptoms were collected and cut into small (2 cm) pieces that were either submerged in water at 16/20, 20/28, 28/33 and 33/40°C (12 h/12 h),respectively, or kept dry at room temperature (control). Moreover, in a field experiment, the stem pieces were placed on the soil surface in a rice field or in a cotton field and either flooded in water or not flooded, respectively. After 1, 2, 4, 6 and 8 weeks, the stem pieces were sampled for retrieval of Lbb on V8-juice agar and for determination of dry weight (Peluola et al., 2013). Selected Lbb isolates from the stem pieces were identified by PCR (Cai et al., 2014).

Results: Results from the two experiments showed that compared to the controls, flooding for 1 to 2 weeks substantially reduced recovery of Lbb and flooding for 4 weeks resulted in negligible recovery of Lbb. All the 99 selected isolates produced a 444-bp DNA fragment in the PCR, confirming that they belong to Lbb. Results also indicated that flooding caused rapid decomposition of the stem pieces. After flooding for 8 weeks, the dry weight of the stem pieces was reduced by 28 to 42% in the laboratory experiment and by 26 to 36% in the field experiment.

Conclusions: Oilseed rape-rice rotation is probably an efficient way to reduce longevity of Lbb in stubble of winter oilseed rape in central China.

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Transcriptomic responses of *Brassica napus L.* to *Leptosphaeria maculans* infection as revealed by RNA sequencing

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Background: *Brassica napus L.* (canola) is one of the most important oilseed crops grown worldwide. Blackleg, caused by the fungal pathogen *Leptosphaeria maculans*, is one of the major constrains for canola production in North America, Australia, Europe and many other regions around the world (Fitt et al., 2006). The disease is mainly controlled by utilization of resistant cultivars, crop rotation and fungicide applications. There is a typical gene for gene interaction between *B. napus* and *L. maculans* at seedling stage. However, *L. maculans* populations can rapidly adapt to selection pressures imposed by single resistance genes and cause resistance breakdown. It's very important to understand host defense mechanisms, especially at the transcriptomic level in the *B. napus-L.* maculans pathosystem to achieve a better control of blackleg. RNA sequencing (RNA-Seq) is an ideal strategy to study transcriptomic responses of *B. napus* to *L. maculans* infection.

Objectives: This study aims to analyse the defense transcriptome profile of *B. napus* in response to *L. maculans* infection using RNA sequencing approach.

Methods: A susceptible canola variety Westar and a canola line DF78 displaying resistance to a *L. maculans* isolate D3 were used for the study. Infected and mock inoculated canola cotyledons were sampled at 3 days post inoculation (dpi), 7 dpi and 11 dpi. Total RNA were extracted from plant samples and RNA sequencing libraries were prepared as described by Kumar et al. (2012) with some modifications. Sequencing was performed on Illumina HiSeq2500 platform. RNA-seq reads were mapped to *B. napus* reference genome. Differentially expressed genes (DEGs) in response to *L. maculans* infection were identified. Functions of DEGs were characterized by homology to *A. thaliana*, with special focus on genes that are reported to be involved in *B. napus-L. maculans* pathosystem or plant defense mechanisms in general. Real-time quantitative PCR (qPCR) was performed to validate expression of a few plant defense genes.

Results: A total of 36 samples were sequenced, with an average of 13.3 M reads per sample. After *L. maculans* infection, number of genes that were upregulated in infected tissues increased over time both in resistant (incompatible interaction) and susceptible (compatible interaction) plants. Gene pattern analysis showed a set of transcripts that were highly expressed only in fungal infected plant samples. A few pathways and a list of candidate transcripts that might have played major roles in defense against *L. maculans* were identified. Compatible and incompatible interactions in this pathosystem showed both common and different defense mechanisms.

Conclusions: RNA-Seq is a promising strategy to discover host transcriptome responses in *B. napus – L. maculans* pathosystem. Defense pathways and transcripts that were involved in this pathosystem in both compatible and incompatible interactions were identified in this study. Results of this study expanded our current understanding of *B. napus* defense responses to *L. maculans* infection.

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397
POSTERS THEME B

Development of clubroot resistant hybrids in omega-9 canola

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Clubroot disease, caused by *Plasmodiophora brassicae*, is a widespread disease that causes serious problems in many *Brassica* growing areas. Symptoms of the disease include formation of galls on the roots of susceptible plants which results in stunting, wilting and even plant death. Clubroot was first discovered in western Canada in 2003 in Alberta, and since then the cases of clubroot infection in the field have been reported increasing and this has become a significant concern to the canola industry in Canada. Due to the fact that resting spores of *P. brassicae* can survive in soil for up to 20 years and no chemical control of the clubroot is currently available, the development of resistant cultivars and cultivation of resistant hybrids is the most efficient way to control this disease. However, resistant sources to clubroot have only been described in European winter canola (*Brassica napus*) and rutabaga as well as in other *Brassica* species. So far up to eight major genes and a few QTLs contributing to clubroot resistance have been reported, but none of these are directly applicable in marker assisted breeding due to various marker platforms used to discover the genes. Omega-9 canola is Dow AgroSciences' proprietary Nexera varieties with high oleic and low linolenic fatty acid composition. Here we present the development of a molecular marker associated with clubroot resistance in *B. napus*, and the development of the first Omega-9 canola hybrids with clubroot resistance.