7th INTERNATIONAL LEGUME ROOT DISEASES Workshop

1st November 2017, East-Lansing, MI, USA

Root diseases are economically important in legumes, affecting large areas of crop production in many countries worldwide. Root rot, caused by *Aphanomyces euteiches*, *Rhizoctonia solani*, *Fusarium* species and wilt, caused by several formae speciales of *Fusarium oxysporum*, are the most destructive soil-borne diseases of pea, chickpea, lentil, faba bean and lupin.

The 7th International Legume Root Diseases (ILRD) workshop is hosted by the BIC/NAPIA 2017 Meeting and held on November 1st, 2017. It followed up previous International workshops on legume root rots held in 2002 (Rennes, France), 2003 (Pasco, WA, USA), 2007 (Rennes, France), 2009 (Pullman, WA, USA), 2014 (Saskatoon, CA) and 2015 (Niagara Falls, CA).

The objectives of the 7th ILRD workshop are to (i) report recent advances of science on root diseases in legumes and (ii)- create a favorable environment for methodology, strategy exchanges and promotion of collaborative networks.
Recent progress of science will cover three major issues, including (i) Pathogen survey, biology, genomics and populations, (ii) Genetics, genomics and breeding for resistance and (iii) Integrated disease management. Exchange sessions will be held about pathogen and plant resources, as well as methodologies and field networks.

Organizers	*Clare Coyne (USDA-ARS, USA)* *Marie-Laure Pilet-Nayel (INRA, France)*
Scientific Organizing Committee	*Sabine Banniza (University of Saskatchewan, Canada)* *Syama Chatterton (AAFC, Canada)* *Anne Moussart (Terres Inovia, France)* *Juan Osorno (NDSU, USA)* *Julie Pasche (NDSU, USA)*

Aphanomyces infected pea root, photo INRA – N. Quéré

Fusarium solani infected pea root, photo Lyndon Porter

Scientific Program

1st November morning, 2017 : Scientific presentations

8:00-8:05 **Clarice Coyne** (USDA-ARS, Washington, USA)
Welcome Introduction

Session 1: Survey, occurrence, pathogen biology, population genetics and genomics
Co-chairs: Sabine Banniza (University of Saskatchewan, Saskatchewan, Canada)
Julie Pasche (North Dakota State University, North Dakota, USA)

8:05-8:35 **Keynote: Christophe Le May** (Agrocampus-Ouest, Rennes, France)
Genetic and phenotypic diversity of pea isolates of *Aphanomyces euteiches* in France and the US. Pg. 6

8:35-8:50 **Telsa Willsey** (Agriculture and Agri-Food Canada, Alberta, Canada)
Detection of interactions between the root rot pathogens *Aphanomyces euteiches* and *Fusarium* spp. Pg. 8

8:50-9:05 **Kimberly Zitnick-Anderson** (North Dakota State University, North Dakota, USA)
Multiplex qPCR assays to quantify *Fusarium* species in root tissue. Pg.9

9:05-9:20 **Samira Safari** (University of Alberta, Alberta, Canada)
Predicting pea root disease using soil DNA analysis and environmental factors. Pg.10

9:20-9:25 **Austin McCoy** (Michigan State University, Michigan, USA)
Phytophthora sansomeana host characterization in popular Michigan crops. Pg. 11

9:25-9:30 **Syama Chatterton** (Agriculture and Agri-Food Canada, Alberta, Canada)
Development of a multiplex digital droplet PCR for quantification of pathogens associated with the pea root rot complex. Pg.12

9:30-10:00 ***Coffee break***

Session 2: Genetics, genomics and breeding for resistance
Co-chairs: Karen Cichy (USDA-ARS, East Lansing, Michigan, USA)
Martin Chilvers (Michigan State University, Michigan, USA)

10:00-10:30 **Keynote: Marie-Laure Pilet-Nayel** (INRA, Rennes, France)
Past, current and future research on genetics of resistance to Aphanomyces root rot of pea. Pg. 14

10:30-10:45 **James Myers** (Oregon State University, Oregon, USA)
Genome Wide Association Study (GWAS) of *Fusarium solani* resistance using the Bean CAP Snap Bean Diversity Panel. Pg. 15

10:45-11:00 **Siu Mui Tsai** (University of Sao Paulo, Sao Paulo, Brazil)
The rhizosphere microbiome as an auxiliary breeding component in common bean against *Fusarium oxysporum.* Pg. 16

11:00-11:15 **Kiela Caudillo-Ruiz** (University of Saskatchewan, Saskatchewan, Canada)
Exploring wild lentil for resistance against *Aphanomyces euteiches.* Pg. 17

11:15-11:20 **Mitchell Roth** (Michigan State University, Michigan, USA)
Root Infection of Soybean (*Glycine max*) and Dry Bean (*Phaseolus vulgaris*) by *Fusarium virguliforme.* Pg. 18

Session 3: Integrated disease management
Co-chairs: Lyndon Porter (USDA-ARS, Washington, USA)
Mary Burrows (Montana State University, Montana, USA)

11:20-11:50 **Keynote: Syama Chatterton** (Agriculture and Agri-Food Canada, Alberta, Canada)
Spatial distribution of *Aphanomyces euteiches* in pea and lentil fields in Saskatchewan. Pg. 20

11:50-12:05 **Anne Moussart** (Terres Inovia, France)
Agricultural practices to prevent and reduce Aphanomyces root rot epidemics and damages on the pea crop in France. Pg. 21

12:05-12:20 **Julie Pasche** (North Dakota State University, North Dakota, USA)
Efficacy of in-furrow fungicides for management of common bean and field pea root rot. Pg. 22

12:20-12:25 **Zachery Noël** (Michigan State University, Michigan, USA)
Managing an oomycete community: fungicide sensitivity and evolution of resistance to ethaboxam. Pg. 23

12:25-12:30 **Viviana Ortiz** (Michigan State University, Michigan, USA)
Temperature adaptation, host specialization and fungicide sensitivity in *Macrophomina phaseolina*, the causal agent of charcoal rot on dry bean and soybean Pg. 24

12:30-12:35 **Kjerstern Oudman** (Michigan State University, Michigan, USA)
Yield loss and the efficacy of fluopyram seed treatment against Fusarium root rot pathogens on dry bean in Michigan. Pg. 25

12:35-14:00 ***Lunch***

1st November afternoon, 2017 : Round Tables

14:00-14:10 **Clarice Coyne** (USDA-ARS, Washington, USA)
Round table introduction

Parallel sessions

14:10-15:30 **Round Table 1:**

- **a- Survey, occurrence, pathogen biology, population genetics and genomics**
- **b- Integrated disease management**

Co-moderators:

- *a- Sabine Banniza (University of Saskatchewan, Canada), Christophe Le May (Agrocampus-Ouest, France)*
- *b- Syama Chatterton (Agriculture and Agri-Food Canada, Canada), Martin Chilvers (Michigan State University, Michigan, USA)*

1/- which scientific questions are shared in the community ?
2/- which tools/resources are available to answer to the common questions ?
Pathogen diagnostic tools; Root pathogen collections

Round Table 2:

- **a- Genetics and genomics of resistance**
- **b- Breeding for resistance**

Co-moderators:

- *a- Clarice Coyne (USDA-ARS, USA), Marie-Laure Pilet-Nayel (INRA, France)*
- *b- James Myers (Oregon State University, USA), Juan Osorno (North Dakota State University, USA)*

1/- which scientific questions are shared in the community ?
2/- which tools/resources are available to answer to the common questions ?
Phenotyping for resistance; Plant genetic material

15:30-16:00 ***Coffee break***

Plenary session

16:00-16:25 Summary-discussion Round Table 1

16:25-16:50 Summary-discussion Round Table 2

16:50-17:00 **Clarice Coyne** (USDA-ARS, Washington, USA)
Marie-Laure Pilet-Nayel (INRA Rennes, France)
Conclusion

Abstract Book

***Session 1*: Survey, occurrence, pathogen biology and population genetics/genomics**

Genetic and phenotypic diversity of pea isolates of *Aphanomyces euteiches* in France and the US

Le May C. [1,2,3], Onfroy C. [1,2], Quillévéré-Hamard A[1,2], Grunwald M.L. 4, Vandemark G[5], Baranger A. [1,2], Pilet-Nayel M-L [1,2], Moussart A. [1,2]

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Aphanomyces euteiches Drechsler is an oomycete pathogen of leguminous crops that causes root rot, a severe disease of pea (*Pisum sativum* L.) worldwide. This homothallic pathogen was reported to attack other legume species (Moussart *et al*., 2008). The genetic and phenotypic diversity of *A. euteiches* populations was studied in different collections.

A collection of 51 French and American isolates recovered from four American (Athena, Le Sueur, Mount Vernon, and Pullman) and three French nurseries (Dijon, Templeux, Riec-sur-Belon) was first studied. Virulence and aggressiveness of the isolates were assessed using two differential host sets of six pea lines and five *Medicago truncatula* accessions. Isolates were genotyped with 20 SSR markers (Mieuzet *et al*., 2016) and SRAP markers (Le May et al., 2017) The pea set revealed two pathotypes (Pathotype I and III), one present in the nurseries of both countries (Pathotype I) and the other (Pathotype III) only observed in some American nurseries (Le Sueur and Athena) (Onfroy *et a*l, submitted). The pea set also revealed that some isolates from Mount Vernon and Athena displayed a similar aggressiveness than French isolates. The *M. truncatula* set allowed the identification of five pathotypes, including one pathotype mainly recovered from French nurseries. Genetic structure of *A. euteiches* populations sampled in the different American and French locations showed low to high genetic diversity within populations, depending on the location (Mieuzet *et al*., 2016; Le May *et al*, 2017). The largest variation occurred within countries, with a total estimated genetic diversity of 0.477 and 0.172 for American and French populations, respectively. Principal component analysis (PCA) and Minimum Spanning Networks (MSN) based on genetic profiles of isolates generated two different clusters, the first one comprising the French isolates and four American isolates (MV1, MV5, MV7, Ath3), and the other one including American isolates.

A collection of 207 isolates from five different French regions was then studied to precise the diversity of French populations of *A. euteiches*. The collection was genotyped using 20 SSR markers and phenotyped for aggressiveness on a set of resistant and susceptible varieties from four legume hosts (pea, faba bean, vetch, alfalfa). Results showed a low genetic diversity of *A. euteiches* French isolates in the collection and no geographical structure, with some isolates from Burgundy showing specific molecular patterns. Variations of aggressiveness were also shown between isolates, as three groups of aggressiveness were identified in the collection.

These studies provided (i) new insights and hypothesesabout major factors shaping the diversity and evolution of *A. euteiches*, such as the role of legume rotation (Barret *et al*., 2008), and (ii) new knowledge and tools for genetic and breeding studies.

Le May C, Onfroy C, Moussart A, Andrivon D, Baranger A, Pilet-Nayel M-L, Vandemark G. 2017. Genetic structure of Aphanomyces euteiches populations sampled from United States and France pea nurseries. *European Journal of plant Pathology*, 10.1007/s10658-017-1274-x

Mieuzet L, Quillévéré A, Pilet ML, LeMay C. 2016. Development and characterization of microsatellite markers for the oomyceta *Aphanomyces euteiches*. *Fungal Genetics and Biology* **91:**1-5.

Moussart A, Even MN, Tivoli B. 2008 Reaction of genotypes from several species of grain and forage legumes to infection with a French pea isolate of the oomycete Aphanomyces euteiches. *European Journal of Plant Pathology* **122**: 321-333.

Onfroy C, Le May C, Tivoli B, Grünwald NJ, Pilet-Nayel M-L, Baranger A, Andrivon D, Moussart A, 2017. Aggressiveness and virulence of *Aphanomyces euteiches* isolates recovered from pea nurseries in the United States and France. *European Journal of plant Pathology* (submitted).

Quillévéré-Hamard A, Le Roy G, Moussart A, Pilet-Nayel M-L, Le May C. 2017. Genetic and phenotypic diversity of pea isolates of *Aphanomyces euteiches* in France. *Frontiers in Plant Sciences* (in preparation)

Barrett LG, Thrall PH, Burdon JJ, Linde CC. 2008. Life history determines genetic structure and evolutionary potential of host-parasite interactions. *Trends in Ecology & Evolution* **23:**678-685

Detection of interactions between the root rot pathogens *Aphanomyces euteiches* and *Fusarium* spp.

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Pea root rot complex (PRRC) describes a group of closely associated soil-borne fungi that cause root rot disease in field pea (*Pisum sativum* L.). The oomycete *Aphanomyces euteiches* Drech. and several species of *Fusarium* are frequently the most prevalent and damaging microorganisms within this complex, causing severe reductions in yield. Fungicide application, crop rotation, and resistance breeding have all been unsuccessful in managing either microorganism thus far. Both pathogen groups have been studied extensively in isolation, but the impact of interactions between *A. euteiches* and *Fusarium* spp. on disease progression remains largely unexplored. To investigate the effect of microbial interactions on root rot severity in pea, greenhouse trials were conducted in which seedlings were exposed to *A. euteiches* and three species of *Fusarium* in varying combinations. For each experimental treatment, an index of disease severity was used to visually rate disease symptoms and pathogen population dynamics were assessed using multiplex quantitative PCR (qPCR). Results from two independent trials indicated an increase in disease severity in the presence of multiple pathogen species compared to single inoculations. Changes in disease severity were confirmed by qPCR results, which revealed significant changes in colonization rates when multiple species were present. These findings suggest that there is an increased risk of significant yield loss in areas where *A. euteiches* and *Fusarium* spp. co-occur, and emphasize the need for integrated crop protection measures.

Multiplex qPCR Assays to Quantify *Fusarium* species in Root Tissue

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Fusarium root rot has caused large yield reductions in field pea across the Northern Great Plains. Among the most important root rotting organisms associated with field pea are numerous *Fusarium* spp. Elucidating which *Fusarium* spp. in the complex are the most destructive is difficult and resource intensive using traditional plating techniques. The goal of this research was to develop hydrolysis probed-based multiplex quantitative PCR assays to quantify biomass of seven *Fusarium* spp. (*F. acuminatum, F. avenaceum, F. culmorum, F. graminearum, F. redolens, F. solani* and *F. sporotrichioides*) commonly associated with field pea root rot. Annealing temperatures, efficiencies and regression coefficients indicate that the resulting simplex assays can be potentially multiplexed in any combination to suit the goals of individual research projects. The relative biomass of seven *Fusarium* spp. quantified from greenhouse inoculated field pea root tissue determined using the multiplexed qPCR assays was similar to the frequency of species identified in plating assays. However, the relative biomass of each *Fusarium* spp. cannot be determined using traditional plating assays. qPCR results indicate that, when co-inoculated under greenhouse conditions, *F. graminearum* colonized host tissue to a greater extent than *F. sporotrichioides*, *F. avenaceum* colonized to a greater extent than *F. culmorum*, and *F. acuminatum* and *F. solani* colonized to a greater extent than *F. redolens*. While this represents only a small number of species combinations, it demonstrates the expansive utility of these assays in elucidating the most important species in the Fusarium root rot complex. The multiplex qPCR assays developed in this research open new avenues of research in the Fusarium root rot complex of field pea, as well as in other host-crops.

Predicting Pea Root Disease Using Soil DNA Analysis and Environmental Factors

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Pea root diseases are considered a growing concern in the Canadian prairies. There is no effective management strategy for this disease; the only proper approach is to avoid planting pea in high risk fields. Therefore developing a model to predict disease risk can be an appropriate strategy. This study was conducted to investigate the possibility of predicting root rot severity under field conditions by using the real time PCR (qPCR) analysis and environmental factors. Two major pathogens associated with pea root rot in Alberta including *Fusarium avenaceum* and *F.solani* f.sp *pisi* were selected for quantification in soil and crop residue by specific qPCR assay. In 2016-2017 growing season, 260 soil and crop residue samples form 20 commercial pea fields across Alberta were collected prior to planting pea in early April and in late June root samples were collected and rated for disease severity. Pearson's correlation procedure was used for pairwise correlation analysis between disease severity and amount of each pathogen DNA (*F. solani* and *F. avenaceum*), rainfall and temperature. The amount of *Fusarium* spp DNA detected by qPCR in pre-seeding soil samples did not show significant correlation with disease severity, which is likely related to low pathogen DNA recovery from soil samples and/or presence of PCR inhibitors in soil DNA extracts. To increase the accuracy of estimation, soil DNA will be tested using droplet digital PCR which is more sensitive and not affected by PCR inhibitors. The results will be used to develop a regression model to predict the disease severity prior to planting. This method requires validation across a wide range of fields with diverse soil types, different cultural practices and a range of different environments.

Phytophthora sansomeana host characterization in popular Michigan crops

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Phytophthora sansomeana is a pathogen of soy and corn, originally differentiated from the *P. megasperma* complex in 1981. Since then, the pathogen has been found in many states within the Midwest, including Michigan. However, little has been done to identify the extent of the host range of *P. sansomeana* in relation to our commodity crops in MI, as well as the Midwest. Corn, soybean and wheat are all popular crops totaling 221 million acres planted within the US in 2016 alone. Similarly, dry bean and sugar beet are popular within Michigan along with winter rye as a cover crop and alfalfa being used as forage. Therefore, we will be examining the host range and pathogenicity of *P. sansomeana* on the previously mentioned seven crops (Corn, soy, wheat, dry bean, sugar beet, winter rye and alfalfa). Without an accurate model of host specificity, crop rotation for disease management may not be an effective means of control. In addition to testing the host range we will also be establishing fungicide sensitivities of *P. sansomeana* to Mefenoxam, Ethaboxam and Oxathiapiprolin. The combination of host range identification and fungicide sensitivities being conducted will allow us to identify better management practices for *P. sansomeana* in Michigan and the Midwest.

Development of a multiplex digital droplet PCR for quantification of pathogens associated with the pea root rot complex

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Root rot of field pea and lentils can be caused by a complex of soilborne organisms. *Aphanomyces euteiches*, *Fusarium avenaceum*, *F. solani* and *F. redolens* were the most frequent pathogens detected during surveys of pea and lentil fields in Alberta, Saskatchewan and Manitoba. Pathogenicity tests indicated that while *A. euteiches* was the most damaging, *Fusarium avenaceum* and *Fusarium solani* were also highly pathogenic to pea, and that combinations of pathogens caused the highest disease. To assess risk of the root rot complex prior to planting peas, an accurate and consistent DNA quantification method to simultaneously assess multiple pathogen levels in soil and relate to disease severity is under development. Previous work indicated that the number of false negatives from field samples containing lower levels of oospore inoculum (~10 – 200 oospore/g soil range) using real-time quantitative PCR (qPCR) was high. Furthermore, when working with the complex matrix of microorganisms in soil samples, the SYBR green PCR method for *A. euteiches* detection also resulted in low levels of amplification of other oomycetes (primarily *Pythium* spp.). Therefore to improve specificity and sensitivity of a quantitative DNA test for soil, a probe-based assay was designed for *A. euteiches* on the digital droplet PCR (ddPCR) platform. The test was then combined with probe-based assays for *F. avenaceum*, *F. solani* and *F. redolens*, and/or an internal plasmid control. The four targets could be separated on the ddPCR platform based on the variation of each targets primer and probe concentrations which changed their respective amplitude wavelength, and clear separation between the targets was achieved. The assay was then applied to root and soil samples, with accurate quantification achieved for diseased root samples. For soil samples, the false negative percentage was improved using ddPCR compared to qPCR, but the protocol needs to be refined further to accurately detect fields with <100 oospores/g soil. The next step is to develop an amended DNA extraction protocol from soil for better extraction efficiency from the target pathogens.

***Session 2*: Genetics, genomics and breeding for resistance**

Past, current and future research on genetics of resistance to Aphanomyces root rot of pea

Pilet-Nayel M-L [1,2*], Coyne CJ [3], Lesné A [1,2], Hamon C [1], Lavaud C [1], Desgroux A [1], Boutet G [1,2], Moussart A [1,2], McGee RJ [4] and Baranger A [1,2]

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Aphanomyces root rot, caused by the oomycete *Aphanomyces euteiches*, is a major soil borne disease of pea in many countries. Genetic resistance is considered a main way to control the disease. Since 2000, INRA has engaged a long-term research program, in close collaboration with USDA-ARS and the support of Terres Inovia, which set up the foundations of research work on pea genetic resistance to *A. euteiches*. The main outputs after 17 years of research include (i) the identification of pea sources carrying partial resistance to *A. euteiches* (Pilet-Nayel et al., 2007; McGee et al, 2012); (ii) the detection of Quantitative Trait Loci (QTL) controlling partial resistance in multiple environments from several RIL populations (Pilet-Nayel et al., 2002, 2005; Hamon et al, 2011, 2013); (iii) the creation of Near Isogenic Lines (NILs) combining one to several QTL in different elite backgrounds, by Back-cross Assisted selection (Lavaud et al., 2015), and the validation of QTL effects in these NILs (Lavaud et al, 2016); (v) the detection of short-size QTL intervals and favourable haplotypes associated with resistance by Genome-Wide-Association Mapping (GWAM) in a pea-Aphanomyces collection (Desgroux et al, 2016).
Current work and prospects include (i) the identification of QTL-closely linked SNP markers, fine mapping of major QTL and validation of underlying candidate genes (ii) the comparative genetic analysis of resistance to *A. euteiches* between major or model legume species (iii) the comparative genetic analysis of resistance to *A.euteiches* and to other stresses in pea, including *Fusarium* sp. (iv) the identification of QTL combinations and QTL pyramiding strategies (Pilet-Nayel et al., 2017) contributing to increase resistance efficiency and durability.

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Hamon C, *et al.* (2013). BMC Plant Biology 13:45
Hamon C, *et al.* (2011). Theor Appl Genet 123:261-281
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Lavaud C, Lesné A, *et al.* (2015). Theor Appl Genet, 128:2273-2288
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Pilet-Nayel M-L, *et al.* (2017). Frontiers in Plant Sci., in revision
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Pilet-Nayel M-L, *et al*. (2002). Theor Appl Genet 106:28-39

Genome Wide Association Study (GWAS) of *Fusarium solani* Resistance using the Bean CAP Snap Bean Diversity Panel

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Root rot diseases are a major constraint to common bean production around the world. Both snap beans and dry beans are affected. Root rot diseases can be caused by a variety of pathogens; however, *Fusarium solani* is a common causal agent. *Fusarium* root rot is a primary yield limitation of snap bean production in Oregon. Cultural control methods are ineffective and the pathogen will be present at the end of one season of production on previously clean land, indicating the need for genetic resistance. In order to address this need, a diversity panel of 148 snap bean cultivars (Bean CAP Snap Bean Diversity Panel) was evaluated for resistance to *Fusarium* root rot in Oregon. Morphological traits including aboveground biomass, adventitious roots, taproot diameter, basal root diameter, deepest root angle, shallowest root angle, root angle average, root angle difference, and root angle geometric mean potentially involved in root rot resistance were also evaluated. Genome-wide association studies were conducted to locate SNPs associated with *Fusarium* root rot resistance QTL and associated morphological traits. Significant associations were located for most traits evaluated, including QTL for root rot resistance on Pv02, Pv03, Pv07, Pv08, and Pv10. With the exception of one QTL on Pv10, the location of these QTL differed from those found in our previously analyzed biparental mapping population based on the OSU5446 (susceptible snap) x RR6950 (resistant dry bean) cross. On Pv08, a SNP marker was associated with both disease resistance and aboveground biomass. A SNP on Pv04 was associated with both basal root and taproot diameter and a SNP on Pv07 was associated with aboveground biomass and several root angle traits. This GWAS study is complementary to previous biparental mapping effort in that it identified many associations with small effect, whereas previous biparental mapping studies tend to identify fewer QTL with larger effect. The genetic factors identified in this GWAS study may not represent resistance genes in the classic sense, but are necessary to achieve high levels of resistance in breeding programs.

The rhizosphere microbiome as an auxiliary breeding component in common bean against *Fusarium oxysporum*

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The rhizosphere microbiome plays a key role in plant growth and health, providing a first line of defense against root infections by soil-borne pathogens. Here, we investigated the composition and metabolic potential of the rhizobacterial community of different common bean (*Phaseolus vulgaris*) cultivars with variable levels of resistance to the fungal root pathogen *Fusarium oxysporum* (*Fox*). For the different bean cultivars grown in two soils with contrasting physicochemical properties and microbial diversity, rhizobacterial abundance was positively correlated with *Fox*-resistance. Pseudomonadaceae, Bacillaceae, Solibacteraceae and Cytophagaceae were more abundant in the rhizosphere of the *Fox*-resistant cultivar. Network analyses showed a modular topology of the rhizosphere microbiome of the *Fox*-resistant cultivar, suggesting a more complex and highly connected bacterial community than in the rhizosphere of the *Fox*-susceptible cultivar. Metagenome analyses further revealed that specific functional traits such as protein secretion systems and biosynthesis genes of antifungal phenazines and rhamnolipids were more abundant in the rhizobacterial community of the *Fox*-resistant cultivar. Metatranscriptome data revealed that community assembly in the rhizosphere follows niche-based mechanisms, presenting lower diversity and distinct community structure comparing to the bulk soil. In comparison with the susceptible plant, the microbiome of the *Fox*-resistant cultivar presented high expression of genes affiliated to the family Paenibacillaceae, a group known by its antifungal activity. The *Fox*-resistant cultivar also presented high expression of genes related to metabolism of nutrients and specific functional traits related to pathogen suppression, such as motility and chemotaxis, and phenazine and colicin V. Network analysis showed similar results to the metagenome approach, revealing a more complex community in the *Fox*-resistant cultivar and pointed the genus *Paenibacillus* as a keystone species in the microbiome. Our findings suggest that breeding for *Fox*-resistance in common bean have co-selected for other unknown plant traits that support a higher abundance of specific beneficial bacterial families in the rhizosphere with functional traits that support a more complex rhizosphere microbiome and reinforce the first line of defense against the pathogen.

Exploring wild lentil for resistance against *Aphanomyces euteiches*

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Aphanomyces root rot (ARR) caused by *Aphanomyces euteiches* was detected in Canadian lentil fields in 2013. Lentil is as susceptible to ARR as pea and no resistance has been found in Canadian lentil cultivars. Wild *Lens* spp. have been recognized as valuable sources of resistance to fungal diseases, hence our objective is to screen 367 accessions of six *Lens* spp. for resistance to ARR. Disease severity is measured as root discolouration ten days after inoculation on a scale from 0 to 5. Results of a preliminary experiment including a few accessions of all wild lentil species (n = 32) revealed that highest resistance to ARR was found in two out of five accessions of *L. culinaris* ssp. *orientalis* and three out of five accessions of *L. lamottei*. Accessions of *L. culinaris* ssp. *orientalis* can be crossed with *L. culinaris* spp. *culinaris*, so these accessions would be valuable sources of resistance.

Root Infection of Soybean (*Glycine max*) and Dry Bean (*Phaseolus vulgaris*) by *Fusarium virguliforme*

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Fusarium virguliforme (Fv) is a soil-borne fungus present throughout the US and is the causal agent of sudden death syndrome (SDS) in soybean. Fv infects roots and causes root rot. Further colonization of the vascular tissues allows Fv to secrete proteins and toxins into the xylem, causing the foliar symptoms of SDS to develop. Other closely related Fusarium species are known to cause root rot in dry beans. Dry bean cultivars from different genetic backgrounds, such as the Meso-American derived 'Zorro' and the Andean-derived 'Red Hawk', are known to show differing levels of susceptibility to root rot. Management strategies to control Fv include using lines with genetic resistance, using fungicide seed treatments, crop rotations, and implementing different tillage techniques. To date, genetic resistance is partial, and few fungicides have shown efficacy against Fv. Crop rotations show mixed effects as Fv can colonize many alternate hosts that may act as a source of inoculum. In this study, we developed a transgenic strain of Fv that overexpresses the green fluorescent protein (GFP) to use as a model to study root infection over time. Information about the infection process may provide insights on how to delay, weaken, or prevent the infections. The soybean cultivar 'Sloan' was pre-germinated for two days prior to inoculation with Fv mycelia. Root cross sections were examined with a fluorescent microscope and indicate that the GFP strain of Fv can colonize the roots, producing dead plant cells that fluoresce red (Figure 1). Additional experiments with various dry bean cultivars will show the degree of susceptibility to Fv infection.

Session 3 **: Integrated disease management**

Spatial distribution of *Aphanomyces euteiches* in pea and lentil fields in Saskatchewan

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Root rot of field pea and lentils causes severe yield losses across the Canadian prairies, where ~4 million hectares of peas and lentils are grown annually. *Aphanomyces euteiches* was first reported in Saskatchewan and Alberta in 2012 and 2013, respectively. Surveys in Alberta, Saskatchewan and Manitoba in 2016 revealed that 40 - 60 % of fields were positive for *A. euteiches*. Owing to its relatively recent detection, the widespread presence of this pathogen throughout the prairies led to further questions regarding its spatial distribution within infested fields. In order to determine the horizontal and vertical distribution of *A. euteiches*, fields that had a history of root rot or showed severe root rot during field surveys were chosen for extensive sampling in the fall. Fields were sampled from all soil zones from Saskatchewan in 2015 – 2017, and soil was collected from 0 – 20, 20 – 40 and 40-60 cm depths along a transect starting in the centre of a diseased patch within each field. Soils were used in a greenhouse bioassay to determine inoculum potential, and an aliquot was also removed for DNA analysis to assess if DNA quantification matched the results of the field survey and greenhouse bioassay. Bioassay results indicated that inoculum potential was highest in the 0 – 20 cm soil layer, but inoculum was present at all depths in most fields. Inoculum was also present at all sites in most fields, suggesting that inoculum is spread uniformly throughout an infested field, with patchy distribution only occurring in a small proportion of the healthier fields. DNA analysis did not always agree with results from the bioassay, with many false negatives occurring in fields with low – moderate inoculum levels. Results demonstrate that *A. euteiches* is well-established in fields cropped to pea and lentil in Saskatchewan, and has likely been present for longer than its recent detection suggests.

Agricultural practices to prevent and reduce Aphanomyces root rot epidemics and damages on the pea crop in France

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Aphanomyces root rot, caused by the oomycete *Aphanomyces euteiches*, is the most important soil borne disease of pea in France. Joint pathology research programs between Terres Inovia and INRA, in the frame of the technological mixed unit PISOM, aim to better understand the disease epidemics and offer solutions to farmers to assess and reduce disease risk in the field. Disease management is primarily based on a biological test used to determine the soil Inoculum Potential (IP) in the plots (Moussart et al., 2009). According to IP levels, advices are given to farmers, based on both (i) the overall opportunity to cultivate pea and if so the choice of the best adapted cultivated type (autumn vs spring sown) (Moussart et al., 2016), in order to prevent yield losses and (ii) the choice of legume species and varieties depending on their genetic resistance and their rate of return in the rotation, in order to prevent IP increase in the plot (Moussart et al., 2008 ; 2013).
Current work and prospects include (i) the development of a molecular test to detect and quantify Aphanomyces inoculum in soil and (ii) the identification of alternative and complementary cultural practices to efficiently reduce soil IP.
The combination of these cultural control practices together with genetic resistance is the key to both preserve soil health and increase resistance efficiency and durability, to control and reduce damages caused by Aphanomyces.

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Efficacy of In-Furrow Fungicides for Management of Common Bean and Field Pea Root Rot

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Root rots of common beans and field peas have caused substantial and widespread yield losses in North Dakota for many years. Root rot symptoms range from small discrete lesions to plant death. Root rot management traditionally has included the use of host resistance, seed treatment fungicides, crop rotation and reducing soil compaction, although these practices also have proven insufficient under high disease pressure. The objectives of this research was to determine the efficacy of in-furrow fungicide applications for management of common bean and field pea root rot under field conditions. Adjacent field trials were conducted from 2015 to 2017 on common beans and field peas, where soil was infested with *R. solani* or *F. solani* and *F. avenaceum* or *F. solani*, respectively. Treatments included QoI, SDHI, and triazole fungicides applied in-furrow, a standard fungicide seed treatment, and non-treated controls, both pathogen infested and non-infested. In nearly every trial, non-treated plots where soil was infested with a pathogen had significantly higher levels of root rot than non-infested soils. Overall, in-furrow treatments significantly improved plant establishment in only a few trials, but generally reduced root rot severity over the seed treatment, although not always significantly. Significant yield increases over the seed treatment were observed with some in-furrow applied fungicides under very high disease pressure. Prothioconazole and penthiopyrad appear to be the most promising fungicides when applied in-furrow for the management of fungal root rots in common bean and field pea, particularly under heavy disease pressure. Although results vary widely between hosts, and across pathogens and disease severity, in-furrow fungicides may provide an additional tool that growers may consider in known high-disease pressure situations and/or when crop value is high.

Managing an oomycete community: fungicide sensitivity and evolution of resistance to ethaboxam

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Seedling diseases caused by oomycetes pose a significant threat to soybean production. In 2011 and 2012, over 80 oomycete species were found to be associated with soybean seedlings. Over half of those species were found to be pathogenic. Evaluation of these species for fungicide sensitivity is important for management. Fungicide amended medium assays are slow, labor intensive and expensive. A high-throughput assay to evaluate fungicide sensitivity of many oomycete isolates at once was developed using optical density measurements of macerated mycelial fragments. Z'-factor was used as a quality control statistic. The assay was utilized to evaluate the sensitivity of 81 oomycete species to mefenoxam and ethaboxam. Of the isolates tested, 87.5% had an $EC_{50} < 1$ μg ml^{-1} and only one *Phytopythium* isolate had an $EC_{50} > 10$ μg ml^{-1} mefenoxam. For ethaboxam, 61.7% of isolates tested had an $EC_{50} < 1$ μg ml^{-1}, whereas, species within *Pythium* clades A, B and E had $EC_{50} \geq 20$ μg ml^{-1} ethaboxam. This suggested that reduced sensitivity to ethaboxam may be inherent and possibly related phylogenetically. Therefore, we investigated the evolutionary history and mechanism of resistance to ethaboxam. Phylogenies indicated that species with reduced sensitivity to ethaboxam followed a convergent evolutionary pattern and had evolved three separate times. Two different transversion mutations lead to the same amino acid change in the target gene of lineages with reduced sensitivity to ethaboxam.

Temperature adaptation, host specialization and fungicide sensitivity in *Macrophomina phaseolina*, the causal agent of charcoal rot on dry bean and soybean

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Macrophomina phaseolina, a soil- and seed-borne fungal pathogen, infects more than 500 plant species and causes charcoal rot disease on dry bean and soybean. Charcoal rot can result in severe yield losses under high temperatures and drought conditions. Therefore, charcoal rot affects soybean production mainly in tropical and subtropical areas, including the southern US and Puerto Rico. However, the incidence of charcoal rot disease in soybean has recently been increasing in the northern US. Temperature adaptation due to pathogen genetic divergence may be involved in the geographically broadening of charcoal rot disease. In this study, 80 *M. phaseolina* isolates, collected from dry bean and soybean grown in the northern and southern US were identified to the species level, characterized for fitness at cold temperature and whole-genome sequenced. All 80 isolates were confirmed as *M. phaseolina* by Maximum Likelihood inference using ITS, EF-1α and ACT loci. In vitro growth rate assays at 15 and 35ºC were used to evaluate the influence of temperature on fungal growth. In the Midwest region, some isolates (M15-4, Md5 and W-25) grew significantly faster at 15ºC than other isolates collected from the same region (p- value <0.05). All isolates were whole-genome sequenced to 20X coverage using a 150 base-pair paired-end strategy on the Illumina HiSeq 4000 platform. Population and comparative genomics will be used to identify genomic regions and candidate genes involved in response to cold temperature and host specialization. Relative mycelial growth of five *M. phaseolina* isolates challenged against Boscalid (SDHI), Iprodione (Dicarboximide) and Prothioconazole (DMI) fungicides were used to identify adequate concentrations for EC50 determinations. Prothioconazole, was initially selected for EC50 determination of a larger set of isolates using 0.01, 0.1, 0.5, 1 and 10 µg ml-1. Initially, 27 *M. phaseolina* isolates were tested and most isolates were sensitive to Prothioconazole. The EC50 distribution had a mean of 0.24, median of 0.21 and range of 0.16 - 1.17 µg ml -1.

Yield Loss and the Efficacy of Fluopyram Seed Treatment against Fusarium Root Rot Pathogens on Dry Bean in Michigan

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Fusarium root rot is a challenge that faces many dry bean growers in Michigan. Root rot causes a reduction in root mass, impeded plant development, and yield loss. Management of root rot has historically relied on cultural management or the use of resistant cultivars, however recently, biological controls and fungicide seed treatments have been used with mixed efficacy. Two *Fusarium* species have become a cause for concern for growers in Michigan. *Fusarium virguliforme*, a member of the *Fusarium solani* species complex (FSSC), causes soybean sudden death syndrome and is prevalent in Michigan soybean and dry bean growing regions. A closely related species, *Fusarium brasiliense*, has been found in recent dry bean root rot surveys in high abundance and is novel in the United States. Despite their prevalence in Michigan, little is known about the effects *F. virguliforme* and *F. brasiliense* on dry bean yield. ILeVO (fluopyram), a seed treatment from Bayer CropScience, has demonstrated efficacy for control of *F. virguliforme* in soybeans but has yet to be tested on dry beans. To determine the impacts of *F. virguliforme* and *F. brasiliense* on dry bean yield and the efficacy of ILeVO seed treatment to protect against root rot, a field trial was conducted in 2017. In the field trial, *F. virguliforme* and *F. brasiliense* inoculum were used in conjunction with a standard seed treatment and a standard plus ILeVO seed treatment on resistant black beans, "Zorro", and susceptible kidney beans, "Red Hawk". *F. brasiliense* was shown to significantly decrease plant mass and lead to high root rot ratings as compared to *F. virguliforme* and the control. Compared to untreated controls, the ILeVO seed treatment significantly increased emergence in the susceptible cultivar and significantly decreased root rot ratings in treated plants.

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