2011 North Central Division Meeting Abstracts

Abstracts presented at the APS North Central Division meeting in Omaha, Nebraska, June 15–17, 2011. The abstracts are arranged alphabetically, by first author's name.

Identification and pathogenic characterization of *Fusarium* spp. on sunflower in the United States

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Phytopathology 101:S2.1

An intensive survey was undertaken in Minnesota, North Dakota, and South Dakota in 2010 to determine the stalk rot pathogens of sunflowers. A total of 1150 diseased stalks from 144 fields were collected, dissected, surface-sterilized, and plated on three types of media: potato dextrose agar (PDA), clarified V-8 juice agar (CV8), and prune yeast lactose agar (PYLA). Plates were scored for presence or absence of different fungi, hyphal tip isolated, and species identified. *Fusarium* was the most frequently isolated fungus (44%). Characterization of *Fusaria* isolates into species was done using morphological identification and analysis of NCBI DNA sequence data for the translation elongation factor 1 α (TEF-1 α) and nrDNA internal transcribed spacer (ITS) genes. Pathogenicity of each species was evaluated in the greenhouse using the stem- and petiole-wound technique. Six 4-week-old sunflower plants (confection cv. CHS RH 3701) at the six to eight leaf growth stage were inoculated by securing mycelia agar plugs of 7-day-old cultures (PDA) with parafilm on stems and petioles wounded with sterile pipette tips. Koch's postulates were completed for each species, external and internal lesion expansion was measured, and pathogen recovery at increasing distances from inoculation points was attempted. More work is in progress to determine the genetic diversity of the *Fusarium* isolates from sunflowers and their associated pathogenicity and aggressiveness.

Characterization of fungicide sensitivity of *Sclerotinia sclerotiorum* isolates from the North Central U.S.

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Phytopathology 101:S2.1

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic plant pathogenic fungus. *S. sclerotiorum* causes Sclerotinia stem rot which is a major disease of canola, although it can also attack many other important crops. This pathogen has a broad geographical distribution, and is prevalent in many US states include those in the North Central region. The absence of genetic resistance makes controlling Sclerotinia diseases difficult, thus farmers depend largely on fungicide applications. Fungicides registered for use against *S. sclerotiorum* include azoxystrobin, pyraclostrobin, thiophanate methyl, boscalid, and metconazole; all of them have single-site mode of action and some also have a history of resistance buildup making the monitoring of fungicide sensitivity a necessity. In this study, the sensitivity of 90 *S. sclerotiorum* isolates to thiophanate methyl was assessed in fungicide-amended potato

dextrose agar. Isolates evaluated had been collected from 12 states in the North Central region of the U.S. EC50 values ranged from 0.01 to 0.88. These values are within the range reported by other researchers and suggest that no resistant strains are present in the sample evaluated. As part of this study, sensitivity to pyraclostrobin, metconazole and azoxystrobin will also be assessed.

Comparison of methods for extracting endoparasitic nematodes from corn roots

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Phytopathology 101:S2.1

The yield of corn in the United States is annually reduced by endoparasitic nematodes. Two endoparasitic nematode genera parasitize corn in the Midwest, *Pratylenchus* spp. and *Hoplolaimus* spp. Previous research has shown nematode population densities to be highly variable and extraction methods may not provide consistent results. In order to determine more consistent results, comparisons were made between three standard nematode extraction techniques for preferences of nematode genera for extraction method, corn root type, and extraction time. The extraction methods evaluated were aeration-incubation, a modified Baermann funnel, and shaker. Research plots were established at two Nebraska locations in 2009 with documented histories of high population densities of these endoparasitic nematode genera. Nematodes were extracted from six root types for 3, 5 and 7 days in a complete factorial treatment design. The percentage of recovery of *Pratylenchus* spp. was greatest after 3–5 days, although extraction rates did not differ statistically between root types and methods until after 7 days. The highest recovery of *Pratylenchus* spp. was found from seminal or first anchor set roots ($p < 0.0001$) using either the aeration-incubation or the Baermann funnel methods ($p < 0.0001$). *Hoplolaimus* spp. were best extracted from either the root fragments on the aeration-incubation ($p < 0.0001$) or from the first set of anchor roots on the Baermann funnel ($p < 0.0001$). This genus had its highest rates of extraction after 5–7 days. For all root types, methods, extraction timings, and nematode genera, the shaker method yielded the fewest nematodes per gram dried root ($p < 0.0001$). Based on these results, it can be concluded that nematode extraction efficiency varies by genus for root type, extraction method, and extraction time required.

Economic analysis of foliar fungicides on soybean in Iowa

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Phytopathology 101:S2.1

The profitability of using foliar fungicides on soybean has not been well studied. As grain prices remain above \$10 per bushel and soybean growers look to maximize profits an economic evaluation of the use of fungicides needs to be done. We evaluated three classes of fungicides: strobilurin, triazole, and strobilurin-triazole premix at 13 site-years across Iowa in 2008, 2009, and 2010. Treatments were evaluated for foliar disease control and yield. Profit (X) was determined using the following equation: $X = (\text{Yield response} \times \text{current price of beans}) - \text{application costs of the treatment}$. The likelihood of growers profiting from fungicides was then determined using Bayesian analysis. Products containing a strobilurin had a higher likelihood of making returns than a product with a triazole alone. We will discuss the

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likelihood of a profitable application for each class, and how the fluctuation of soybean prices affects the likelihood of a grower making a profit.

Variation of *Triticum mosaic virus* (TriMV) titer in winter wheat infected by TriMV alone and in combination with *Wheat streak mosaic virus*

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Phytopathology 101:S2.2

Triticum mosaic virus (TriMV) is a recent addition to the virus complex in wheat. It is transmitted by the wheat curl mite (WCM) (*Aceria tosichella*), which also transmits *Wheat streak mosaic virus* (WSMV) and *High plains virus*. Cultivar resistance has been documented for WSMV, but not for TriMV. TriMV titer was monitored in a WSMV-susceptible (Millennium) and a WSMV-resistant (Mace) cultivar mechanically inoculated with TriMV or TriMV+WSMV in the field and greenhouse. Inoculated plants were sampled every 10 days in the field throughout fall, and at 28 days after inoculation (DAI) in the greenhouse. Virus titer was determined using quantitative ELISA. Relative chlorophyll content was measured with a SPAD meter each sampling time. In the field, TriMV titer increased over time in Millennium but remained below detectable levels in Mace. In the greenhouse, TriMV was detected in TriMV- and WSMV+TriMV-inoculated plants in both cultivars, but the titer was higher in Millennium than in Mace. Millennium inoculated with TriMV+WSMV had the lowest SPAD readings both in the field and greenhouse. In Mace, SPAD readings did not differ between treatments, but were higher than in Millennium both in the field and greenhouse. TriMV titer had a significant inverse linear relationship with SPAD readings only in Millennium both in the field (slope = -0.25, $R^2 = 74.43\%$, $P = 0.0013$), and greenhouse (slope = -0.54, $R^2 = 71.10\%$, $P = 0.0002$). These results indicate that the WSMV-resistant cv. Mace is also resistant to TriMV and that TriMV titer suppression is greater under low field temperatures.

Characterization of quorum sensing and cyclic-di-GMP signaling systems in *Erwinia amylovora*

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Phytopathology 101:S2.2

The enterobacterium *Erwinia amylovora* is a vascular pathogen that causes fire blight. Determinants of pathogenicity have been characterized, including the secretion of effector and harpin proteins through the Type III Secretion System, and the production of the exopolysaccharide amylovan and biofilm formation. As demonstrated in other plant pathogens, the expression of some virulence factors during infection is usually controlled via Quorum Sensing (QS) signaling systems. We used comparative genomic analyses to search homologs of genes involved in QS regulation. The results suggest that the *E. amylovora* genome harbors several genes corresponding to the Autoinducer-3/Epinephrine/Norepinephrine signaling system in *E. coli*. Particularly, the putative homologs to the two component system QseEF and the transcriptional regulator QseA are highly conserved in *E. amylovora*. Gene-knockout experiments revealed that the QseEF_{EA} system is involved in the regulation of swarming motility, biosynthesis of amylovan and biofilm formation. In addition, *E. amylovora* encodes eight genes involved in the biosynthesis and/or degradation of cyclic-di-GMP (c-di-GMP), a second messenger signaling compound. Individual knockout mutations in each of these genes revealed that c-di-GMP positively regulated biofilm formation and negatively regulated motility. These results demonstrated that *E. amylovora* possesses several regulatory networks that govern the expression of virulence genes.

Evolution of mating type genes: Heterothallism is ancestral in the *Didymellaceae*

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Phytopathology 101:S2.2

Fungi within the *Didymellaceae* including anamorphic *Phoma* and *Ascochyta* spp. cause economically significant diseases on a range of agronomic and floriculture crops including, pea, chickpea, lentil, pyrethrum and chrysanthemum. Mating type gene organization and function plays a significant role in the epidemiology and biology of fungi. To characterize the function and evolution of mating type genes within the *Didymellaceae*, we cloned and characterized these genes of two sets of closely related sister species; *D.*

pinodes and *A. pinodella* both pathogens of pea, and *P. ligulicola* var. *ligulicola* and *P. ligulicola* var. *inoxydabilis* primarily reported as pathogens of chrysanthemum and pyrethrum (both members of the Asteraceae), respectively. Each pair of sister species contains a known homothallic and a possible heterothallic species as demonstrated by pure culture phenotypes, i.e. single spore isolates of the homothallic species routinely produce a teleomorph in pure culture. As predicted homothallic isolates contained both mating type genes at a single locus, while putative heterothallic isolates only contained a single mating type gene. We cloned mating type genes MAT1 and MAT2 from isolates of *A. pinodella*, however we were only able to clone a single mating type gene MAT1 from isolates of *P. ligulicola* var. *inoxydabilis*. Mating type gene organization was conserved amongst the heterothallic species, including the previously reported MAT genes for *A. rabiei* and *A. lentis*, with a putative pyridoxamine phosphate oxidase (PPO) upstream of MAT genes and putative DNA lyase downstream. Orientation of MAT genes within homothallic *P. ligulicola* var. *ligulicola* was conserved, with MAT1 being downstream of MAT2. However, mating type gene organization in homothallic species *D. pinodes* was not conserved with the inversion of MAT genes. This finding provides evidence that the heterothallic state is conserved and ancestral to the homothallic state within the *Didymellaceae*.

Distribution and impact of sudden death syndrome in Minnesota

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Phytopathology 101:S2.2

Sudden death syndrome (SDS) caused by *Fusarium virguliforme* ('Fv') is a threat to soybean production. This disease was first reported in Minnesota (MN) in 2002 in two southeastern counties. SDS had been confirmed by 2009 in 23 counties across southern and central MN. The objectives of our studies in 2010 were to determine: (i) the distribution of SDS in MN, (ii) if new isolates of 'Fv' vary in virulence, and (iii) the effects of SDS on yield of susceptible and partially resistant soybean varieties. A survey conducted across MN soybean growing regions included over 400 plants from 97 fields with foliar symptoms similar to SDS. Preliminary diagnosis based on symptoms (root rot, interveinal chlorosis and necrosis on leaves, discoloration in lower stem) indicated that plants in 65 fields had SDS. 'Fv' was detected in plants from all 65 fields using a specific PCR assay, resulting in confirmation of SDS in 14 additional counties and bringing the total to 37 counties. Seven isolates of 'Fv' obtained from plants in six counties were tested for virulence in a greenhouse. Virulence varied among these isolates based on root and leaf symptoms and effects on plant biomass for inoculated soybean varieties susceptible ('S') and partially resistant ('PR') to SDS. In replicated field studies using a moderately virulent isolate of 'Fv', yield of an SDS-'S' variety was reduced 31% in plots with SDS compared to plots without SDS, and yield of the 'S' variety was 20% lower than the 'PR' variety in plots with SDS. SDS appears to be an increasing disease in MN with potential to cause large soybean yield losses.

Sunflower hybrid susceptibility and the impact on yield to sunflower rust in North Dakota

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Phytopathology 101:S2.2

Two types of sunflowers (*Helianthus annuus*) are grown in North Dakota; confections, which are used for direct consumption, and oilseed, which are used for oil. Sunflower rust, caused by *Puccinia helianthi* (Schwein), has emerged as an important yield-limiting disease in North Dakota sunflower production. Incidence of infected fields and fields with rust severity above the fungicide-action threshold (3%) has increased throughout the 2000's. The objectives of this study were to evaluate rust resistance in commercial hybrids and the impact of rust on yield. Rust severity and yield were assessed in field trials in 2009 and 2010 at NDSU's Carrington Research Extension Center in Carrington, ND. Rust severity in excess of 3% was observed on 89% of confection hybrids in both years, with 56% of the hybrids in 2009 having a severity in excess of 20% in 2009. Rust severity in excess of 3% was observed on 53% and 2% of oilseed hybrids in 2009 and 2010, respectively. Eleven percent of oilseed hybrids had severity in excess of 10% severity in 2009. In 2009, the yield range was approximately 500 lb/A to 1,800 lb/A in confections and 400 lb/A to 2,200 lb/A in oilseeds. Correlation between rust severity and yield was significant in both confection ($r = -0.689$, $P = 0.0016$) and oilseed ($r = -0.639$, $P = 0.0001$) hybrids in 2009. Yield was compromised by a sunflower midge epidemic in 2010. The aggregate virulence phenotype of *P. helianthi* field-isolates collected in trials was race 336; the most commonly reported race in recent North Dakota surveys. Results indicate the majority of sunflower hybrids in North Dakota are susceptible to

rust, and severity is strongly correlated to yield reduction. Greater rust severities were observed more frequently in confection hybrids than oilseed hybrids, but yield reductions were similar, indicating that oilseeds may be more sensitive to rust.

Field evaluation of *Rhizoctonia solani* - *Heterodera glycines* interactions on soybean

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Phytopathology 101:S2.3

Soybean cyst nematode (SCN; *Heterodera glycines*) is involved in disease interactions with other soilborne pathogens of soybean (*Glycine max*). The soilborne fungal pathogen *Rhizoctonia solani* interacts with nematodes in crops other than soybean; however, little is known about the potential interactions of SCN and *R. solani* on soybean. Field studies were conducted in Urbana, IL in 2009 and 2010 to evaluate the effects of both SCN and *R. solani* on soybean. The studies were conducted with a SCN-susceptible and a resistant cultivar in fields with high SCN population densities. Soil of half the plots was augmented with sterilized grain sorghum colonized with *R. solani*. In addition, soybean seeds were treated with either mefenoxam (to control oomycete pathogens only); mefenoxam + A16148 (to control *R. solani*); mefenoxam + abamectin (to control SCN); or mefenoxam + A16148 + abamectin. In both years, seeds with a treatment that included A16148 resulted in significantly ($P = 0.05$) greater plant stands compared with other treatments. In the *R. solani*-infested areas in 2009, yield of the SCN-susceptible cultivar treated with mefenoxam + A16148 + abamectin was reduced by 19% compared to the areas not infested with *R. solani*. Conversely, yield of the SCN-resistant cultivar treated with mefenoxam + A16148 + abamectin was reduced by only 1% in the *R. solani*-infested area compared with those in the non-infested area. This indicates that SCN-susceptible cultivars may be affected more severely by *R. solani* than SCN-resistant cultivars under high SCN pressure. Additional studies of this disease interaction are underway.

Broad spectrum virus resistance in plants using the Oligoadenylate synthetase (OAS) pathway

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Phytopathology 101:S2.3

Oligoadenylate synthetase (OAS) pathway is an animal antiviral pathway that was utilized to develop broad spectrum virus resistance in plants. The pathway is composed of two enzymes, a 2,5A synthetase that produces 5'-phosphorylated, 2',5'-linked oligoadenylates (2-5A) upon activation by viral dsRNA, and a 2-5A-dependent RNase L. Here we evaluated the suitability of the pathway in tobacco (*N. tabacum* and *N. benthamiana*), soybeans and wheat plants. Tobacco plants expressing the transgenes showed resistance to Tobacco mosaic virus (TMV), Tobacco etch virus (TEV) and Cucumber mosaic virus (CMV D and Y strains). Similarly, transgenic soybean carrying the OAS system was also evaluated for resistance against Soybean mosaic virus (SMV) and Bean pod mosaic virus (BPMV) and transgenic wheat against Wheat streak mosaic virus (WSMV). These results showed that the system has the potential to provide broad spectrum resistance against different viruses and can confer resistance to different crops.

Rapid detection of fungicide resistance in *Venturia inaequalis*

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Phytopathology 101:S2.3

Venturia inaequalis is a fungal pathogen that causes the disease apple scab on apple and crabapple trees (*Malus* sp.) and can result in severe yield losses and defoliation of the tree. Fungicides are the primary means of scab control, and many of the fungicides used for management of apple scab are site-specific, meaning they target one biochemical process. As such, these single-site specific fungicides are more prone to causing resistance within the pathogen population. The demethylase-inhibitors (DMI) are a class of single-site fungicides; in *V. inaequalis*, resistance was found correlated to promoter mutations that lead to the over-expression of the CYP51A1 gene. Primer sets were developed to examine over 32 isolates of *V. inaequalis* from Indiana and Michigan that were classified resistant or sensitive to further evaluate the relationship between the role of the upstream insertion and fungicide resistance. To date, we have found resistant isolates with larger amplification bands consisting of the insertion or mutations and sensitive isolates with smaller amplification bands consisting of no insertions or mutations.

The effects of light-weight rolling on dollar spot disease in creeping bentgrass putting greens

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Phytopathology 101:S2.3

Dollar spot (*Rutstroemia floccosum* syn. *Sclerotinia homoeocarpa* F.T. Bernet) is one of the most important diseases to affect golf course turf grasses in the United States. A 3-yr study to investigate the effects of light-weight rolling on the suppression of dollar spot was conducted with the objective of elucidating disease reduction mechanisms. Creeping bentgrass (*Agrostis stolonifera* L.) plots subjected to daily (5 days wk⁻¹) rolling treatments (1x a.m., 1x p.m., 2x a.m.) showed significant disease reductions when compared to a non-rolled control. Treatments rolled in the afternoon (after dew and guttation fluid dissipated) exhibited similar disease reductions as treatments rolled in the morning. The 2x day⁻¹ treatment exhibited significantly less dollar spot than all other treatments ($P < 0.05$), as well as significantly higher rootzone volumetric water content (VWC) than the control. Microbial populations were evaluated via phospholipid fatty acid (PLFA) analysis in order to investigate potential effects of rolling on microbial abundance and dynamics in the upper root zone. Rolled treatments exhibited significant increases in PLFA abundances related to common bacteria, as well as a general trend towards increased total bacterial biomarker abundance when compared to the non-rolled control. Results indicate added physical and biological effects of rolling on turfgrass, which may be contributing to dollar spot disease suppression.

Sampling and detection efficiencies of the U.S. and Canadian Plum pox virus eradication programs

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Phytopathology 101:S2.3

Plum pox virus (PPV) was first detected in the U.S. in Pennsylvania in 1999 and in Ontario, Canada in 2000. Following the implementation of a ten-year PPV survey and eradication program, PPV was officially declared eradicated in Pennsylvania in 2009. Despite the implementation of a similar (but less stringent) eradication program in Canada in 2000, PPV has yet to be eradicated in Ontario. The U.S. and Canadian Eradication Programs, though similar, differ in a number of important ways. These include: (i) the number of leaves sampled and tested per tree, (ii) the ELISA test kit used to test Prunus leaves, and (iii) the removal (eradication) protocols that are enforced when a PPV-positive tree is detected. The goal of this research, therefore, was to compare how the U.S. and Canadian PPV Eradication Programs affect the probability of detecting PPV at the leaf, scaffold, tree, and block spatial scales. A simulation model was developed to quantify and compare the detection efficiency of the U.S. versus Canadian protocols for detecting PPV. The sampling and detection protocols used in the U.S. (tree scale) had a detection efficiency of 72%, compared to 41% for Canada's. Employing a stratified (by scaffold) random sampling design did not improve PPV detection efficiency compared to using a simple random sampling design to sample leaves from Prunus trees. Detection efficiency increased with increasing sample size (leaves/tree), however, gains in detection efficiency diminished as sample size increased. There was good agreement between the ELISA kits used in the U.S. and Canadian at the leaf and scaffold scales (based upon Chi-square analysis), but not at the tree scale. The results from this research have important implications with regards to the two Eradication Programs, and their impacts on successfully detecting and eradicating present and future outbreaks of PPV.

Emergence of new virulent races of *Plasmopara halstedii* inciting downy mildew on sunflower in the United States

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Phytopathology 101:S2.3

Prior to 2009, no isolate of *Plasmopara halstedii*, the cause of sunflower downy mildew (DM), recovered from sunflower in the United States caused disease on resistance genes Pl₆, Pl₇ or Pl₈. These genes, originally released by the USDA-ARS Sunflower Research Unit in 1985, have been used extensively to produce downy mildew-resistant (DMR) hybrids. In 2009, downy mildew was observed in a field of DMR sunflowers located in Bottineau county (north central) North Dakota. The sample was inoculated on a standard set of nine differential lines and categorized as race 734, and conferred virulence on the USDA line HA-335 which contains the Pl₆ gene. In response, 49 isolates of *P. halstedii* were collected in a subsequent survey and virulence-phenotyped. Fourteen of 49 DM samples infected HA-335. In 2010, 17 of 160 isolates collected during surveys infected HA-335. These 31 isolates

conferring virulence on Pl_6 were identified as five races, namely 314, 704, 714, 734 and 774. Isolates virulent on Pl_6 were predominantly from North Dakota, but also found in Minnesota and Nebraska; none were recovered from South Dakota. Race 714 comprised approximately one-half of the 31 samples. To determine resistance in commercial hybrids, eighty DMR hybrids were tested in greenhouse trials with the five races. Only 17 hybrids were immune to all races. Thus, nearly 80% of commercial hybrids marketed in the United States as “DMR” are susceptible to the new races, presumably since these hybrids rely on either the Pl_6 or Pl_7 genes. None of the five newly identified races overcome the Pl_8 gene in USDA line RHA-340, nor the Pl_{arg} gene in USDA lines RHA-419 and RHA-420. The identification of races conferring virulence on one of the most utilized resistance genes in commercial sunflower may facilitate the re-emergence of DM as a significant yield threat.

Identification of exotic alleles that confer native resistance to western corn rootworm beetles

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Phytopathology 101:S2.4

Western corn rootworms (WCR) are one of the most damaging pests of maize, causing substantial global economic losses due to diminished yield and control costs. Despite widespread presence of WCR in the corn belt, U.S. corn has been protected by conventional means such as crop rotation, as well as by insecticidal transgenes. However, rotation resistant WCR populations have evolved independently at least twice, and transgenic crop failures have been reported in each of the past several years. Significant research efforts to improve the efficacy and epidemiological durability of insecticidal transgenes is ongoing, but there is also a need to identify and deploy native resistance (NR) mechanisms. A principle challenge remains identifying and characterizing the effects of such natural alleles, so that the eventual goal of understanding the underlying mechanism(s) can be met. Through repeated evaluations of diverse germplasm accessions, two exotic landrace varieties were identified with enhanced tolerance to WCR. F2 and backcross populations were generated separately for each exotic source for quantitative trait loci (QTL) mapping. These segregating populations were evaluated on several measures of resistance over two nursery seasons. Genotypes were collected for 170 SNPs distributed across the ten maize chromosomes. Several significant associations between genotype and phenotype were detected in both our F2 and BC1 populations. These findings demonstrate that allelic differences in the host confer varying levels of native resistance, and further, that these functional polymorphisms are experimentally tractable. We discuss the challenges for pursuing fine-scale mapping and detailed allele characterization using this below-ground plant-pest interaction system.

Managing Botrytis gray mold of geraniums with biological fungicides

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Phytopathology 101:S2.4

The amount of pesticides used annually by the agriculture industry is nearly a billion pounds, worth over US \$31 billion. According to the USDA, biological fungicides are beneficial fungi and bacteria that attack and control plant pathogens. *Botrytis cinerea* causes blight, a very common and destructive disease of greenhouse crops that is estimated to cause the largest economic loss of all ornamental diseases. Cease is a biological fungicide with the active ingredient *Bacillus subtilis* Strain QST 713, a ubiquitous naturally occurring bacterium that controls many plant fungal pathogens. The common practice recommended by plant pathologists of using different modes of action was incorporated into an experiment for controlling *B. cinerea* with a biological fungicide. Veranda O has the active ingredient of polyoxin D zinc salt, a byproduct of the soil bacterium *Streptomyces cacaovii* var. *asoensis*. The mode of action is to interfere with the fungal cell wall production by inhibiting chitin synthase. In this experiment, both biological fungicides were used alone and in alternating combinations, each starting with a different mode of action. Fungicides were sprayed once per week for four weeks inoculating with a spore suspension four hours later. Plants were bagged to maintain high humidity. Neither Cease nor Veranda O used alone gave significant disease control. Using alternating combinations of the two biological fungicides on a weekly basis significantly reduced the sporulation of *B. cinerea*. The Cease/Veranda O combination gave the highest level of disease control when compared to the other treatments. The combination starting with Cease and then Veranda O on a weekly basis provided a comparable level of control to a fungicide commonly used by the greenhouse industry to manage botrytis gray mold. This data set shows that rotations between different types of biological fungicides may be suitable alternatives to conventional fungicides.

Mycoparasites of *Sclerotinia sclerotiorum* a fungus that causes soybean white mold

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Phytopathology 101:S2.4

Soybean white mold is caused by *Sclerotinia sclerotiorum*. Sclerotia of this fungus remain viable in the soil for several years, and disintegration of sclerotia is difficult with fungicides. Therefore, alternatively fungi mycoparasitising sclerotia play significant role. Therefore, we investigated fungi that were mycoparasitising sclerotia in our collection of 1995–2010 from naturally infected soybean plants, Iowa. Twenty randomly selected sclerotia from each year were surface sterilized and placed on vermiculite (5 Sclerotia per plate). Weekly observations for five months did not show any apothecia production but we observed degeneration of sclerotia. The identity of the mycoparasitising fungi were confirmed following morphological and molecular techniques. The fungi that were disintegrating some of the sclerotia were (1) *Coniothyrium minitans* Strain N09 (GenBank Accession No. HQ908274), (2) *Aspergillus fumigatus* strain AF/IA/N07 (GenBank Accession No. JF729022) and (3) *Talaromyces trachyspermus* strain TT/IA/N05 (GenBank Accession No. JF714646). Further studies on the growth rate, inhibition and degeneration abilities of these mycoparasites appear to have potential bio-control ability against *S. sclerotiorum*.

Differences between *Coniothyrium minitans* (CM) of commercial grade Contans® WG and N09 - a new strain of CM from Iowa

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Phytopathology 101:S2.4

Contans® WG is a biological control product marketed for control of *Sclerotinia*. This product contains *Coniothyrium minitans* (CM), a mycoparasitic fungus. While evaluating sclerotia collection at Iowa State University, new strain N09 of CM (CMN09) mycoparasitising sclerotia was identified. Attempts were made testing differences between the strains. (a) Seven days incubation on PDA at 23°C, CMN09 growth was 6-mm more than Contans and after 30 days, pycnidiospores production in CMN09 was 1½ times more than Contans, (b) Inhibition ability of the strains was assessed placing 6-mm diameter blotter discs dipped in pycnidiospores suspension of the strains, and placing a disc of a strain on one end and surface sterilized sclerotium on the other end on PDA plate, the growth rate of CMN09 was higher and the sclerotia formation was lower than that of Contans (c) degeneration efficiency of sclerotia was assessed by spraying individual spore suspension of the strains on sclerotia placed in glass Petri dishes containing un/sterilized soils, and incubated at 23°C and 3°C, sclerotia from each treatment was sampled at 15 days interval for 90 days and plated on PDA for viability. Sclerotia sprayed with CMN09 had low to 0% viability in sterilized soils compared with Contans and controls, indicating CMN09 may be more aggressive than CM in Contans.

Field evaluation for bacterial leaf blight resistance in spring wheat germplasm

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Phytopathology 101:S2.4

Pseudomonas syringae, the causal agent of wheat bacterial leaf blight was reported four decades ago in South Dakota and nearby states. Host resistance could potentially be an effective and durable method for controlling this disease since chemical sprays are not practical for bacterial disease management. The objective of this experiment was to identify bacterial blight resistant spring wheat germplasm. Forty-five hard red spring wheat genotypes with different genetic backgrounds were evaluated for resistance to bacterial blight in field with supplementary inoculation of *P. syringae* pv. *syringae*. Field experiments were carried out in Codington and Brookings Counties in 2009 and 2010. Disease severity was assessed using double digit (00-99) disease rating scale after heading through dough stage at 7-day intervals. Considerable variation in degree of resistance among the genotypes was clearly observed. A continuous distribution of disease severity observed in each year showed that resistance against the disease is most likely governed by many genes. Combined analysis showed that SD 4148 was least infected and followed by Faller, Knudson, and Alsen, whereas SD 4176, SD 4171, RUSS, SD 4011, and SD 3948 were highly infected. Although significant genotype by location interaction was present, fourteen genotypes (SD 4148, Faller, Knudson, Alsen, SD 4199, SD 4024, SD 4023, Reeder, SD 4178, SD 4144, SD 3997, SD 4159, SD 4119, and SD 4187) out of forty-five tested, had consistently lower disease severity than average. The findings of this study provide the evidence of genetic resistance which will be further investigated in QTL mapping studies and development of improved wheat cultivars.

Viruses detected in North Dakota dry edible pea nursery plots

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Phytopathology 101:S2.5

North Dakota ranks number one in dry edible pea production in the U.S. with 405,000 of 837,900 total U.S. acres in 2010. In 2009 and 2010, plants with virus-like symptoms were observed at multiple locations across ND, collected, and tested using commercially available ELISA kits for the following four viruses: *Bean leaf roll virus* (BLRV), *Pea enation mosaic virus* (PEMV), *Pea mosaic virus* (PeMV), and *Pea streak virus* (PeSV). In 2009, 55 samples were only collected from breeder nurseries. In 2010, 51 and 18 samples were collected from breeder nurseries and commercial fields, respectively. In 2009 both leaves and pods were tested individually, but in 2010, only the foliage was tested. Symptomatic samples typically reacted with one or more of the antisera in 2009. Of these, 29, 9, 39, and 7 samples were positive in 2009 for BLRV, PeSV, PEMV, and PeMV, respectively; viruses were not detected in 10 samples. Mixed infections were detected with 19, 15, 9, and 2 samples being co-infected with 1, 2, 3, and 4 viruses respectively. In 2010, viruses detected in 7 samples from breeder plots included 4 BLRV alone, 1 BLRV + PeSV, 1 PeSV alone, and 1 PEMV alone. No viruses were detected in samples from commercial fields in 2010. This is the first report of viruses that infect dry edible pea in North Dakota. However, more extensive surveys need to be conducted to determine the incidence and severity of these viruses in commercial fields.

Arabidopsis NDR1 is an integrin-like protein with a role in fluid loss and plasma membrane-cell wall adhesion

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Phytopathology 101:S2.5

Arabidopsis thaliana NDR1 (NON-RACE SPECIFIC DISEASE RESISTANCE-1) is a plasma membrane localized protein, which is required for disease resistance mediated by several CC-NB-LRR class resistance proteins including RPS2, RPM1 and RPS5. Loss of NDR1 leaves a plant with increased susceptibility to the bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000 in both a PAMP (PATHOGEN ASSOCIATED MOLECULAR PATTERN) dependent as well as an effector dependent manner. While the genetic requirement for NDR1 in plant defense signaling has been previously established, the physiological role for NDR1 had remained elusive. Our study focused on determining the global physiological role for NDR1 utilizing an integrated approach. Homology modeling and structure threading revealed a high degree of structural homology to *Arabidopsis* LEA14, an abiotic stress associated protein, as well as mammalian integrins, well characterized membrane localized proteins involved in adhesion and stress signaling. The *ndr1-1* mutant plants demonstrated a substantial alteration in induced electrolyte leakage in response to the *P. syringae* effector AvrRpt2. Along with an alteration in induced leakage observed in the mutant line, NDR1 was found to be required for mediating the plasma membrane-cell wall adhesion through an RGD (*Arg-Gly-Asp*)-like motif. Furthermore, the requirement for NDR1 in PTI (PAMP TRIGGERED IMMUNITY) was analyzed revealing an attenuated signaling response to the flagellin subunit flg22 in the *ndr1-1* mutant. Together these data indicate a broad role for NDR1 in mediating defense signaling through the regulation of electrolyte leakage by maintaining the plasma membrane-cell wall connection.

Fungicide sensitivity of *Colletotrichum* isolates from an outbreak in an Indiana apple orchard

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Phytopathology 101:S2.5

Bitter rot of apples, caused by the fungal pathogens *Colletotrichum gloeosporioides* and *C. acutatum*, is a significant late-season disease of commercial apple. Strict quality standards for the fresh fruit market require orchards to make multiple fungicide applications per season to control fruit rots. In the summer of 2010, several orchards reported outbreaks of bitter rot despite multiple applications of fungicides. Isolates were collected from infected fruit from two Indiana apple orchards to determine what role, if any, fungicide resistance played in this failure. A mycelial growth assay was used to assess the sensitivity of 30 isolates to kresoxim-methyl, thiophanate-methyl, and fenbuconazole. These chemicals represent three Fungicide Resistance Action Committee (FRAC) groups with moderate to high risk of resistance development. Fenbuconazole inhibited all mycelial growth into the media, even at the lowest concentration. The growth radius of isolates from a sprayed orchard grown on kresoxim-methyl and thiophanate methyl amended media was statistically equal to those from an unsprayed orchard (data not

shown). These preliminary results indicate no shift in fungicide sensitivity for kresoxim-methyl, thiophanate-methyl, and fenbuconazole.

Evaluation of commercially available tests for the diagnosis of Goss's bacterial wilt and blight in corn

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Phytopathology 101:S2.5

Forty-four isolates of *Clavibacter michiganensis* subspecies *nebraskensis* (Cmm; causal agent of Goss's wilt) and 40 symptomatic foliar samples from fields were tested with lateral flow chromatographs (ImmunoStrips®; Agdia, Inc., Elkhart, IN). A subset of these isolates were tested with ELISAs developed for the detection of Cmm, *C. michiganensis* subsp. *tesellarius* (Cmt), *C. michiganensis* subsp. *sepedonicus* (Agdia, Inc., Elkhart, IN) and Cmn (Neogen, Inc., Scotland, UK). The GEN III OmniLog Identification system (Biolog, Inc., Hayward, CA) was also evaluated, with limited success, for its ability to identify Cmn isolates propagated on three different media types. The highest identification percentage for Cmn was achieved when Biolog Universal Growth (BUG™; Biolog, Inc., Hayward CA) agar without blood was used to propagate isolates prior to inoculation of the microarray plate. Cmn isolates were positively identified in all of the tests conducted with ImmunoStrips as well as with the Cmn, Cmm and Cmt ELISAs. The results of this study suggest that the ImmunoStrips developed for the detection of Cmm, as well as ELISAs developed for the detection of Cmn, Cmm, and Cmt can be used with confidence to detect Cmn.

Research solutions in a non-model system: Developing tools to understand sugar beet-*Fusarium oxysporum* interactions

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Phytopathology 101:S2.5

Fusarium yellows of sugar beet (*Beta vulgaris*), caused by *Fusarium oxysporum* f. sp. *betae* (*Fob*), is a problem for sugar beet production regions. Little is known about how *Fob* infects sugar beet roots to elicit disease. Additionally, a high rate of non-pathogenic *F. oxysporum* (*Fo*) are also commonly recovered from symptomatic plants. The ability to better understand pathogenicity will be beneficial for future experiments to characterize *Fusarium* yellows of sugar beet. The major objective of this project was to generate fluorescently labeled *Fob* and *Fo* isolates, using an *Agrobacterium tumefaciens* mediated transformation with both a green fluorescent protein (ZsGreen) and red fluorescent protein (rfp) for development of *in-situ* microscopy methodologies to characterize the mode of interaction of *Fob* and *Fo* with sugar beet roots. Twelve *Fob* and *Fo* isolates were successfully transformed with ZsGreen. Of these, one *Fob* and one *Fo* isolate were tested for pathogenicity with no changes due to the transformation event. Confirmation of putative rfp transformants is currently underway. This research developed new tools that can now be used to characterize early stage infection process during *Fusarium* yellows in sugar beet.

Impact of wheat streak mosaic virus on spring inoculated winter wheat lines

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Phytopathology 101:S2.5

Wheat streak mosaic virus (WSMV; Family: *Potyviridae*; Genus: *Tritomovirus*) causes the most prevalent viral disease in South Dakota wheat. Fall WSMV transmission by wheat curl mites (*Aceria tosichella* Keifer) to emerging wheat is the classic infection pattern in South Dakota. However, a second infection pattern also occurs when wheat curl mites, moving from primary infection sites in winter wheat or from overwintering on alternate hosts, transmit WSMV to winter wheat during spring growth. This second pattern is typically considered only a minor cause of loss in winter wheat. The 2010 WSMV Winter Wheat Evaluation Nursery was inoculated during the spring rather than the fall due to inclement weather conditions. Thus, the data collected models the second infection pattern. Yields ($p < 0.0001$) and test weights ($p < 0.0001$) were significantly affected by spring WSMV inoculations. Yield losses ranged from 0–30.7%, and losses in test weight ranged from 0–20%. Thus, spring infection by WSMV is not a minor source of loss in some winter wheat lines. Fifteen winter wheat lines were identified with minimal losses for both characters. These included Alice, Fuller, Hatcher, Hawken, Overland, SD06W156-5, SD07W053, SD08080, SD08133, SD08177, SD08196, SD08198, Smoky Hill, Wendy-W, and Wesley. These lines can form the basis for future recommendations in situations where spring infection is likely to occur.

Sensitivity of Michigan populations of *Monilinia fructicola* to sterol demethylation inhibitor fungicides and analysis of *CYP51* promoter insertions

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Phytopathology 101:S2.6

The major fungal pathogen affecting Michigan cherry and peach fruit is *Monilinia fructicola*, causal agent of American brown rot (ABR). The most effective class of fungicides for ABR management are the sterol demethylation inhibitors (DMIs). Recent publications have suggested that a PCR-based survey that detects a novel 376-bp insert, 'Mona', upstream of the cytochrome demethylase gene (*CYP51*; target site of DMIs), can also be used to detect DMI-resistant isolates. In the southeastern United States, DMI resistance has been attributed to this upstream insert, causing overexpression of the *CYP51* gene. Traditionally, relative growth (RG) assays on DMI-amended medium are used to detect DMI-resistant isolates of *M. fructicola*. Between 2008 and 2010, we sampled a total of 69 commercial orchards in Michigan and 905 and 369 isolates were examined for DMI phenotype using the RG and PCR methods, respectively. The in vitro assay detected only one resistant isolate and three orchard populations shifting toward resistance. Amplification of the promoter region upstream of *CYP51*, however, resulted in the detection of DNA insertions in a wide range of isolates. Sequence analysis of the inserts from random DMI sensitive, shifted, and resistant isolates revealed the presence of the 'Mona' sequence, or other unique sequences. Further genetic investigation is needed to determine the function of these inserts in Michigan isolates and their relation to DMI phenotype.

Simulated moisture regimes developed to assess rainfastness and efficacy of Dithane® formulations

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Phytopathology 101:S2.6

Foliar-applied fungicides in the field are exposed to a range of moisture conditions including dew, rain, mist, fog, and overhead irrigation. Moisture can have a large impact on performance by influencing fungicide wash-off, redistribution on the leaf surface and even degradation rate. Polymers can be added to formulations to improve fungicide adherence to foliage surfaces and resist degradative effects of moisture events. A method was needed to assess the suitability of a variety of formulation additives to enable fungicide resistance to different moisture regimes. The objective was to develop methodology to determine the effect of different moisture regimes on the performance of Dithane® formulations, and to be able to detect performance differences among Dithane® DGNT (Dispersable Granule Neo Tec) and other candidate formulations. The methods used included simulation of 8 different moisture regimes (combination of timing of moisture, and type of moisture, dew or rain). Exposure to any of the 8 moisture regimes prior to inoculation caused a significant ($P < 0.1$) reduction in efficacy of the 3 formulations evaluated compared to no moisture before inoculation. The greatest difference in efficacy among the 3 Dithane® formulations was observed with the rain plus dew moisture regime applied 4 hours after formulation application. Although not significant under all regimes there was a trend for the Dithane® WP plus experimental polymer X formulation to have greater efficacy than Dithane® DGNT which was greater than Dithane® WP (wetable powder).

Validation of alternative sources of weather data as inputs to a warning system for sooty blotch and flyspeck of apple

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Phytopathology 101:S2.6

Sooty blotch and flyspeck (SBFS) is a complex of epiphytic fungi that infest apple and other tree and vine fruit crops. In regions with humid climates, SBFS fungi blemish the fruit cuticle, reducing market value of the crop. In 2010, a warning system (WS) for SBFS in the Upper Midwest U.S. was evaluated in an Iowa apple orchard. Objectives were to compare sources of weather data as inputs, and evaluate the warning system's performance when strobilurin fungicides were used. The WS input cumulative hours of relative humidity (RH) > 97%. Once 192 hours of RH > 97% is reached, a calendar-based spray program begins. RH was measured within the tree canopy using Spectrum WatchDog sensors. Alternatively, RH was estimated remotely using a web-based decision tool. Pyraclostrobin plus boscalid (Pristine) and trifloxystrobin (Flint) were compared to captan tank mixed with thiophanate-methyl (Topsin M 70WP). Control treatments were a calendar-based (biweekly) spray using captan tank mixed with thiophanate-methyl and no fungicide sprays after first cover. Using on-site RH measurements, the WS saved 3 sprays compared to the calendar-based treatment, while providing equivalent control of SBFS. Strobilurin-containing fungicides controlled SBFS as effectively as the thiophanate-methyl plus captan tank mix. The web-

based decision tool severely underestimated hours of RH > 97%, resulting in a SBFS control failure.

First report of sooty blotch and flyspeck fungi on cucurbit crop hosts

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Phytopathology 101:S2.6

Sooty blotch and flyspeck (SBFS) is a complex of more than 60 species of epiphytic fungi that colonize the epicuticular wax layer of the fruit of apple, pear, persimmon, plum, and other crops. On apple, SBFS signs become visible mid-to late summer as surface blemishes, reducing the value of fresh market fruit in humid regions worldwide. In this study, SBFS-infested squash (*Cucurbita maxima*, cv. Blue Hubbard) were collected from fields at the Iowa State University Horticulture Research Station (ISUHRS) in Gilbert, Iowa. In October 2009, colonies of SBFS-like fungi were excised with subtyping squash cuticle, pressed, photographed, and isolated onto acidified water agar. Of 44 primary isolates from 65 peels sampled, 16 pure cultures were selected for further examination. Partial sequences of the ITS region (ITS1, 5.8S rDNA and ITS2) were compared to known species of fungi using BLAST. Four putative species were delineated using parsimony analysis: two *Dissocoonium* spp., a *Diatriactium* sp., and a *Scleroramularia* sp. In July 2010, a modified version of Koch's postulates was conducted on cv. Golden Delicious at the ISUHRS to verify that these fungi could infest apple fruit. After harvest, DNA was extracted from excised apple peels and ITS sequences matched those of the original squash isolates. To our knowledge, this is the first report of SBFS fungi on cucurbit hosts and the first confirmation that fungi from squash can produce SBFS signs on apple fruit.

Differential transmission of Triticum mosaic virus across wheat curl mite (*Aceria tosichella* K.) colonies collected within the Great Plains

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Phytopathology 101:S2.6

Wheat is an important food grain worldwide, and it is the primary dryland crop in the western Great Plains. A complex of three viruses (wheat streak mosaic, high plains, and triticum mosaic viruses) is a serious cause of loss in winter wheat production in the Great Plains with an estimated average annual loss of about 2%. Triticum mosaic virus (TriMV) was only recently identified in Kansas in 2006. A survey conducted on wheat fields with symptomatic plants in the Great Plains indicates that the frequency of TriMV is unpredictable between growing seasons. Greenhouse studies indicate that co-infections of TriMV and wheat streak mosaic virus induce more severe symptoms than either virus alone, increasing the potential impact on wheat yields. Currently, three colonies of WCM have been characterized by differential responses to mite resistant genes (biotypes) in wheat and differential transmission of high plains virus. A study was designed to determine TriMV transmission for these various wheat curl mite colonies. Avirulent wheat curl mites from each colony were reared on TriMV infected and virus-free wheat plants. For each source plant, individual mites were transferred to 10 separate test plants and virus transmission determined via ELISA assay. Source plants were replicated 18 times for each treatment (180 individual mite transfers). Results indicate that TriMV is only transmitted by one of the three wheat curl mite colonies. This is the same mite population that was found to differentially transmit High plains virus. The results from this study demonstrate the importance of the mite source on virus epidemiology and the potential impact of this virus complex in the Great Plains.

Fluctuations in wheat diseases in North Dakota, 2004–2010

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Phytopathology 101:S2.6

North Dakota State University's (NDSU) Integrated Pest Management (IPM) survey of wheat diseases began in 1988 with one field scout hired to identify wheat leaf and head diseases in eastern North Dakota (ND). Survey results from 1988–2003 were previously reported. Currently, the program has five seasonal scouts to cover 3.5+ million hectares (predominately hard red spring wheat) in 53 counties. This report covers the fluctuations in disease occurrence from 2004 to 2010. The number of fields surveyed per year ranged from 810 to 1471, approximately one field per 2500 to 4000 hectares planted. Tan spot was the most consistent disease identified among 17 diseases assessed, with an average detection in 70% of fields surveyed. Tan spot caused an estimated 3.4% yield loss per year, based on prevalence and

severity. Fusarium head blight and the Septoria leaf disease complex were severe in 2005, likely favored by the warm humid conditions that year, but in the other six years, detections and severities were low. Wheat leaf rust occurrence fluctuated, with highest detection in 2005 (28% of fields) and lowest detections (2–7% of fields) from 2008 to 2010. Loose smut was common most years (occurrence in 5–30% of post heading fields). Generally, stripe rust, spot blotch, bacterial stripe, barley yellow dwarf, or wheat streak mosaic were observed in less than 10% of surveyed fields per year. Flag smut, Cephalosporium stripe, and dwarf bunt were not detected any year. Survey data gave extension and research scientists, producers, and the wheat industry a weekly snapshot of the presence, distribution, and severity of diseases. This information was immediately provided to wheat producers, via the NDSU Crop and Pest Report, the NDSU IPM web page, the AgDakota listserve, and county Ag Alerts, to help them assess their current and future management needs.

Sunflower rust fungicide timing trial in Nebraska 2010

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Phytopathology 101:S2.7

In 2010, the United States planted 1.8 million acres of sunflower (*Helianthus annuus*). In the U.S., sunflower is grown for both oil or for human consumption along with lower quality seeds are included in birdfeed. The most prevalent and damaging disease in sunflower in Nebraska is rust, caused by the fungal pathogen *Puccinia helianthi*. This pathogen can infect and survive on both wild and cultivated sunflower. Rust has been increasing in severity within the last 8–10 years, and until recently, few labeled fungicides were available for use by the sunflower growers. Because of the development of several new fungicides for managing this disease and the uncertainty for optimal times for application, a study was conducted in 2010 in Nebraska with the purpose of evaluating the effect of pyraclostrobin (Headline) fungicide application timing based on plant growth stages for maximizing yield and reducing disease control. Results in 2011 indicated that fungicide applications provided better protection for reducing disease incidence and severity when made in the R4-R6 plant growth stages. Yield results for this study were not significantly influenced for any application timing, presumably due to the later onset and development of disease.

Dispersal of *Sclerotinia sclerotiorum* in birdseed

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Phytopathology 101:S2.7

Sclerotinia sclerotiorum, the cause of white mold, is a fungal pathogen that infects over 400 species of plants including crops such as soybean, dry bean, and sunflower. There is very little host resistance to *S. sclerotiorum* and fungicides are expensive. Thus, sunflower producers often have some infection in their crop. In sunflower processing facilities when the amount of sclerotia and infected seeds among the harvested seed exceeds 2%, the seeds, which were originally targeted for oil or food, are sold for birdseed. This increases the probability that *S. sclerotiorum* is present in birdseed mixes and is distributed across the U.S.A. An isolate recovered from a birdfeeder with sunflower seeds in Western Nebraska was found to be clonal with a Mycelial Compatibility Group (MCG) of 3 isolates from the sunflower processing facilities in Minnesota and North Dakota and also to an MCG of 13 geographically diverse dry bean isolates. In addition, a comparison of isolates from sclerotia sampled from the sunflower processing facilities and bean isolates demonstrated clonality between 5 of these birdseed isolates and MCGs from geographically widespread and diverse bean isolates. These results demonstrate that this commercial practice has likely facilitated the dispersal of *S. sclerotiorum*. It is also of note that near the birdfeeder from which the previously mentioned isolate was taken, an *S. sclerotiorum* infected zinnia was also found. Although the isolate taken from the zinnia was not found to be clonal to the birdfeeder isolate, the close proximity of both support the possibility that this practice has the potential to spread the pathogen to new regions.

Evaluation of fungicide efficacy and timing for management of common bean rust in North Dakota

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Phytopathology 101:S2.7

Region-wide epidemics of common bean rust (*Uromyces appendiculatus* Pers.:Pers.) in North Dakota's dry edible bean crops (*Phaseolus vulgaris* L.), have been absent since the deployment of the rust resistance gene *Ur-3* in the mid 1990's. In 2008, a race of rust conferring virulence to *Ur-3* was detected for the first time in North Dakota, rendering the North Dakota crop susceptible. In response, fungicide efficacy and fungicide timing trials were conducted at three locations in North Dakota in 2009 and 2010. To establish disease in these trials, but avoid impacting nearby commercial fields, trial plots were planted to a pinto bean cultivar lacking the *Ur-3* gene (GTS-900) and inoculated with urediniospores of *U. appendiculatus* isolate known to be avirulent to *Ur-3*. Ten leaves on each plot were visually-evaluated 4–5 times throughout the season to estimate pustule coverage. Area Under Disease Progress Curve (AUDPC) and relative Area Under Disease Pressure Curve (rAUDPC) were calculated for each location. All fungicides applied in the efficacy trial reduced the AUDPC from the non-treated check and some AUDPC differences among fungicides were observed. AUDPC differences among discrete timings were also observed, but in general, sequential application timings showed the greatest decrease in rust severity.

Rapid molecular detection method for *Pyrenophora tritici-repentis* isolates insensitive to QoI fungicides

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Phytopathology 101:S2.7

Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is an important disease affecting wheat worldwide. Quinone outside inhibitors (QoIs) have been applied to manage tan spot in wheat; however, QoIs target only the cytochrome b (cyt b) site in complex III of mitochondria and thus pose a serious risk for resistance development. The resistance mechanism to QoIs is due to point mutations in the cyt b gene. The main objective of this study was to develop a molecular detection method for the four known mutations responsible for the shift in the sensitivity to QoI in the field isolates of Ptr. Twelve specific primers designed to clone a portion of the cyt b gene with known mutations in order to serve as controls since QoI insensitive isolates of Ptr have not been reported in the United States. The partial cyt b gene clones were sequenced to identify nucleotide substitution at G143A and F129L sites. Genomic DNA of the mutated partial cyt b gene clones and the European QoI insensitive and reduced sensitive isolates of Ptr possessing G143A and F129L were amplified by polymerase chain reaction (PCR) using two primer pairs and further digested with three restriction enzymes (BsaJI, Fnu4HI and MnlI). The results of digested PCR product of known insensitive and reduced sensitive isolates of Ptr had DNA signals for mutation GCT at G143A and mutations TTA, TTG and CTC at F129L. The amplified region at the F129 had 99% sequence similarity with *P. teres*, the net blotch pathogen of barley. We further tested reduced sensitive isolates of *P. teres* possessing mutations TTA and CTC at F129L and results showed identical DNA bands to the partial mutant cyt b clone. This newly developed two-step molecular detection method is rapid, robust, and specific to monitor QoI insensitive and reduced sensitive isolates of Ptr.

Identification of QTL contributing tan spot resistance to a novel isolate of *Pyrenophora tritici-repentis* in spring wheat

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Tan spot, caused by *Pyrenophora tritici-repentis* (Ptr), is an important foliar disease in wheat. Ptr is classified into eight races based on their ability to induce tan necrosis and chlorosis on wheat differentials. Recently, disease reactions similar to race I has been found on wheat differentials when inoculated with a group of isolates of Ptr collected from winter wheat from Arkansas. These isolates lack the ToxA gene, which was confirmed previously by multiplex PCR and Southern blot assays. In this study, 535 spring wheat accessions from different regions around the world were inoculated with isolate AR CrossB10, a representative of novel Ptr isolates from Arkansas, and the association mapping (AM) approach was used to identify quantitative trait loci (QTL) involved in resistance to the AR CrossB10. These wheat accessions were tested with 832 polymorphic

diversity array Technology (DArT) markers. Association analysis using two QK mixed models identified 11 QTL responsible for resistance to AR CrossB10 and located on chromosomes 1A, 1D, 2B, 2D, 6A and 7A. These QTL together accounted for 14.4% of the phenotypic variation. A QTL resistance to extensive chlorosis was previously reported on chromosome 1A. The remaining seven QTL found in the present study are apparently novel. Pyramiding novel QTL along with toxin insensitivity genes into an adapted wheat cultivar could provide broad-spectrum resistance to Ptr.

Field mortality of soybean cyst nematode *Heterodera glycines* populations in soybean-corn rotation systems in Nebraska

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Soybean cyst nematode (SCN) is the most yield-limiting disease of soybean in the North Central Region of the U.S. A study was established in Nebraska to assess the mortality of SCN populations in soybean production fields annually rotated to corn. Twenty two fields in soybean-corn rotation and under different water (irrigated and rain-fed) and tillage regimes were selected in 2009 in the major soybean-producing areas of the state. In each field, ten 3 × 3-m sampling grids were selected systematically in a zig-zag pattern and the center of each grid was georeferenced with a handheld GPS device for site-specific resampling. From each grid, 20 individual soil cores were collected and mixed to obtain a single composite sample. Sampling was conducted in early spring 2009, preceding corn planting, and in early spring 2010, following corn harvest. Soil samples were processed in the laboratory to determine the number of SCN eggs per cm³ of soil. Preliminary data showed that in this rotation year SCN population mortality in the sampled fields ranged from 0 to 91.7% with an average decline of 57%. Cluster analysis identified at least three different groups of fields based on pH, organic matter content, textural composition, and the SCN percentage population change. The observed difference in population declines in tilled vs. non-tilled fields in this rotation year was inconsistent.

The GATA factor *Asd4* regulates the TOR response to nitrogen deficiency and is essential for virulence in the devastating rice blast pathogen

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Phytopathology 101:S2.8

Magnaporthe oryzae is arguably a devastating rice pathogen. Tps1 is its glucose sensor and a regulator of the infection controlling the transition from appressorium development to infectious hyphal growth in the plant. Its activity is mediated by three Nmr repressor proteins, which regulate the activity of GATA-family transcription factors. Yeast-two hybrid analysis demonstrated physical interactions between the Nmrs and at least three GATA transcription factors Nut1, Asd4 and Pas1. Gene functional analysis, using the split marker strategy, revealed Asd4 is essential for pathogenicity. In addition, Asd4 was an important regulator of growth on repressing nitrogen sources (e.g. ammonium, glutamine, and asparagine). Using the TOR inhibitor, rapamycin, the wild type strain shows attenuated growth on minimal media. While Δtps1 mutant strains were insensitive to rapamycin compared with the wild type, the Δasd4 mutant strain had attenuated growth even in the absence of rapamycin. TOR is an important eukaryotic kinase pathway, which plays an essential role in the cell response to environmental nutrient status. Thus, the response of the TOR pathway to nitrogen repressing conditions is dependent on a GATA transcription factor, Asd4, and is essential for appressorium development and pathogenicity.

Effects of pyraclostrobin foliar fungicide and harvest timing on corn stalk health

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The use of foliar-applied fungicides on corn (*Zea mays*) in the United States has increased drastically in recent years. In addition to managing foliar diseases, foliar fungicides have been marketed as increasing stalk health of corn. Field trials were conducted in Urbana, IL in 2008, 2009, and 2010 to determine the effect of pyraclostrobin fungicide on corn stalk health. In the trials, four corn hybrids were planted. Corn hybrids 'H-8838' and 'H-8939' were moderately susceptible to gray leaf spot (GLS; caused by *Cercospora zeae-maydis*) and 'H-8852' and 'H-8953' were moderately resistant to GLS. In addition, 'H-8838' and 'H-8939' were "sister" lines, as well as 'H-8852' and 'H-8953', with 'H-8939' and 'H-8953' containing transgenic *Bt* traits for corn borer and rootworm control. Plots were either treated with pyraclostrobin fungicide at the R1 growth stage or left non-treated. In addition, plots were harvested either "on-time" or 3 to 4 weeks later. Stalk health was evaluated immediately prior to harvest by splitting the stalks open and visually rating

stalk rot using a 0 to 5 severity scale. In general, corn hybrids that were moderately susceptible to GLS had greater stalk rot severity than those that were moderately resistant to GLS. Within "sister" lines, no stalk rot differences were observed between *Bt* and non-*Bt* hybrids. Stalk rot severity generally was greater at the later harvest dates compared to the "on-time" harvest dates. In general, plots treated with pyraclostrobin had reduced stalk rot severity compared with those left non-treated.

Effect of foliar-applied pesticides and surfactants on ear development in hybrid dent corn

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In recent years, growers have dramatically increased the amount of foliar-applied pesticides to hybrid corn (*Zea mays* L.). These applications are intended to increase yield, however reports of a unique phenomenon known as arrested ear development (AED) have also increased. At maturity, arrested ears have a shorter cob length, reduced kernel number, and a brown cob remnant at the tip. Fields with AED symptoms were often sprayed with pre-tassel applications of pesticides and surfactants, including fungicides. A research trial was established in West Lafayette, IN in 2009 and 2010 to identify contributing factors to arrested ear development in hybrid corn, and determine the optimum timing for foliar-applied pesticides on hybrid corn to prevent ear damage and subsequent yield loss. Treatments included applications of fungicides, herbicides, insecticides, a nonionic surfactant (NIS) spray adjuvant. Treatments were applied alone and in combinations, at V14 and VT growth stages. Ten ears per plot were collected and ear arrest incidence and severity were measured. Application timing had a significant effect on ear arrest ($P = <.0001$ for 2009 and 2010). Pesticide treatment also had a significant effect on ear arrest ($P = 0.0073$ and $P = <.0001$ for 2009 and 2010, respectively). Treatments containing an NIS adjuvant resulted in a significantly higher percent of arrested ears than other treatments at the same application timing.

Collection of Cochliobolus sativus isolates and assessment of virulence pattern

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Cochliobolus sativus (anamorph: Bipolaris sorokiniana), the causal organism of common root rot (CRR) and leaf spot blotch (SB) diseases in wheat, is an economically important fungal pathogen globally. Different pathotypes of C. sativus have been reported the U.S.A. and elsewhere and information on pathogen population structure have potential implications for disease management and varietal development. Two field surveys, southern and northern South Dakota, were performed in summer 2010 to collect Cochliobolus sativus isolates and to assess its virulence patterns from different parts each at least 10 km distant. The virulence pattern of these isolates was determined in the greenhouse using three cultivars of each wheat, barley and oat. Inoculation was done 12–14 days after planting and rating was done 9–12 days after inoculation based on the infection responses from host plants by using 1–9 rating scale where 1–4 being resistant and above 4 as a susceptible. From southern SD, Cochliobolus was isolated from 22 out of 35 fields, and 29 out of 42 fields in northern SD. Results indicate that the interaction between isolates and cultivars is highly significant. Six virulence groups (VG) were identified where VG 0.0.0 was the least virulent group and VG 7.0.0 was the most virulent. VG 6.4.0 is only one group that infected barley line ND-5883. None of the VG infected the oat lines used in this study. The result suggests divergence among C. sativus isolates population from South Dakota.

Soybean sudden death syndrome in Michigan

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Phytopathology 101:S2.8

Soybean sudden death syndrome (SDS), a potential yield limiting disease has been reported from all major soybean producing regions in North and South America. In the United States, *F. virguliforme* has been reported as the predominant pathogen. *F. virguliforme* is a soil-borne fungus that infects soybean roots and produces toxins that cause foliar symptoms. In Michigan, *F. virguliforme* may have been present for a number of years; however its presence was only recently confirmed. In 2009 and 2010, twelve counties with SDS symptomatic plants were confirmed to have *F. virguliforme*. Since soybean SDS is relatively new to Michigan, we are actively mapping to

county level the distribution of *F. virguliforme* across Michigan to understand the disease distribution and spread. Although soybean cyst nematode (SCN) is not required for SDS development, high levels of SCN may increase disease severity. In this study, soil and plant samples were collected from a soybean germplasm evaluation trial to examine the interaction of SDS development with *F. virguliforme* quantity, soil fertility and nematode counts. Soil and soybean roots were processed to perform dilution plating enumerations on modified Nash Snyder media (MNSM) for *F. virguliforme*. Data analysis suggests there is a weak positive but not statistically significant correlation between DX (disease index) and the quantity of *F. virguliforme* and SCN in the field samples. Similar to previous studies available K (potassium) in the rhizosphere soil was significantly ($P < 0.01$) positively correlated with DX. Due to the limitations of traditional plating methods, we are currently developing a real-time qPCR assay to rapidly and reliably detect and quantify *F. virguliforme* in plant and environmental samples.

Development of two soybean cyst nematode (*Heterodera glycines*) populations from Nebraska on henbit (*Lamium amplexicaule*) under controlled conditions

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Phytopathology 101:S2.9

Soybean cyst nematode (SCN) is the most yield limiting disease of soybeans in the United States. Reported as an alternative host for the SCN, henbit is a winter annual weed species that has become prevalent in non-tilled lands in the North Central region of the U.S. A greenhouse study was conducted to evaluate the development of two different SCN populations from Nebraska on henbit at three post-inoculation times. The treatment design was a factorial with two SCN populations, Neligh (HG type 2.5.7) and West Point (HG type 7), and three harvesting times (14, 21, and 28 days after inoculation with SCN eggs), with 6 replications per treatment combination. Henbit seeds were germinated and then transplanted to individual pots filled with 750 ml of sterilized soil. Pots were placed in a water bath bench that kept soil temperature constant during the study. Seven days after transplanting, pots were inoculated with 2,000 SCN eggs from each population. At each harvesting time, plant roots were stained and the number of J2, J3-J4, males, and attached cysts per plant were counted. Soil was processed to determine the number of cysts detached from the roots. Plant shoot and root dry weights were recorded. The number of cysts per plant at the last harvesting time, and shoot and root biomass of the henbit plants infected by the populations did not differ (P -value = 0.75, 0.62 and 0.69, respectively). The pattern of SCN development on henbit was different for the two populations at the three harvesting times indicating that the Neligh population reproduced faster than the West Point population. These results suggest that some populations of SCN may infect henbit roots earlier and develop more rapidly than others. Further studies to understand the influence of henbit removal timing on SCN populations and their possible interactions are underway.

Effects of temperature on growth and aggressiveness of *Sclerotinia homoeocarpa*

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Phytopathology 101:S2.9

Dollar spot, caused by *Sclerotinia homoeocarpa*, is an important disease of most turfgrass species worldwide. *S. homoeocarpa* was described almost a century ago by F.T. Bennett, however the basic biology and epidemiology of the pathosystem is still unclear. Four isolates of *S. homoeocarpa* from Wisconsin and six isolates from Oklahoma were grown on native soil and USGA greens-grade sand. WI isolates were grown with and without creeping bentgrass (CRB) debris and incubated at temperatures ranging from 11 to 34°C. OK isolates were grown with CRB debris only at temperatures of 15, 20, 25, 30, and 35°C. Radial growth of mycelia was recorded at 24, 48, 72, and 96 hours past infestation. Growth for all isolates was most rapid between 20 and 30°C. WI isolates grew best on native silt loam with CRB debris, while the effect of soil treatment was not significant for the OK isolates. Growth was limited and sporadic at temperatures below 15°C. To assess aggressiveness, CRB plants were inoculated with 3 WI isolates and 6 OK isolates and placed in separate growth chambers set at 10, 14, 20, 25, 30 or 34°C. Disease severity was visually assessed every 24 hours for four days. Disease developed in each temperature treatment, yet was most severe at 20 and 25°C for all isolates. These data indicate a temperature window for dollar spot development, but also demonstrate that another environmental parameter such as relative humidity is likely more important for dollar spot development.

Optimizing the efficacy and environmental fitness of a commercial *Pseudomonas* bacterial biocontrol product for the control of turfgrass disease

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Phytopathology 101:S2.9

Dollar spot caused by *Rutstroemia floccosum* syn. *Sclerotinia homoeocarpa* F. T. Bennet, is the most costly disease on golf courses. The 6-8 fungicide sprays that are required to control this disease can present a hazard to golfers, maintenance personnel, and the environment. This pathogen is becoming resistant to many different fungicide chemical classes thus increasing the need for development of a sound biocontrol alternative to chemical fungicides. A fungicide/antibiotic-producing bacterial strain of *Pseudomonas aureofaciens* Tx-1 (Tx-1) has been commercialized for golf course use with some success in recent years. To achieve adequate control it must be applied daily. This may be partially explained by UV susceptibility of Tx-1, which is a common limitation of biocontrol agents. Results from various wavelengths of ultraviolet radiation (A, B, C) exposure tests showed a significant drop in Tx-1 survivors following increased dosage. Protection with a promising sunscreen was demonstrated in all UV exposure tests. Addition of this sunscreen to Tx-1 cultures and the highly UV-B sensitive mutant *Pseudomonas aeruginosa* (phl-, uvrA-) provide statistically significant improvement in UV-B tolerance compared to Tx-1 or *P. aeruginosa* alone. In an attempt to identify the most UV-resistant individual cells, Tx-1 was exposed to intensive UV light (UV-C) for various lengths of time (in vitro). Cells (10TC30) with significantly improved survival rate after 7 J/m² UV-C irradiation was found followed by 10 cycles of 30 seconds of UVC exposure. Results from bioassays against *R. floccosum* showed the production of antibiotics efficacious did not change after UV exposures. Field data of dollar spot studies showed that applied at high concentration (107CFU/cm²), Tx-1 sunscreen mixture and the strain 10TC30 provided promising disease control on the field study. Population sizes of the treatments were monitored, and results demonstrate the improved percentage of survival of Tx-1 with the protection of sunscreen.

Field resistance of *Cercospora sojina* to QoI fungicides and management with alternative fungicide chemistries

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Frogeye leaf spot (FLS), caused by *Cercospora sojina*, causes yield reductions of soybean (*Glycine max*) grown in the United States and other countries. A primary method of managing FLS is the use of foliar fungicides in the quinone outside inhibitor (QoI) class. Because QoI fungicides have a high risk of fungal pathogens developing resistance to them, a fungicide resistance monitoring program was established for *C. sojina* which included the determination of baseline sensitivities to QoI fungicides. In 2010, *C. sojina* isolates were collected from soybean fields treated with QoI fungicides from several states. Isolates collected from two locations in Illinois, one location in Kentucky, and two locations in Tennessee were found to have QoI fungicide sensitivity levels that were over 200-fold higher than baseline isolates using petri dish assays. A greenhouse trial was conducted with a QoI-resistant isolate from Tennessee and a QoI-sensitive isolate that is part of the baseline isolate collection to: i) confirm that the putative QoI-resistant isolates could cause FLS on leaves treated with QoI fungicides; and ii) determine if fungicides with other modes of action could control FLS caused by the QoI-resistant isolates. In the greenhouse trial, FLS caused by the QoI-resistant isolate was not significantly ($P \geq 0.05$) reduced with QoI fungicides compared to a water control, but FLS caused by the QoI-sensitive isolate was significantly reduced with QoI fungicides compared to a water control. Several fungicides in the demethylation inhibitor (DMI) group and thiophanate methyl (a methyl benzimidazole carbamate, MBC, fungicide) significantly reduced FLS caused by the QoI-resistant or QoI-sensitive isolate compared to their respective water controls. These results indicate that *C. sojina* isolates resistant to QoI fungicides are present in Illinois, Kentucky, and Tennessee, and that FLS caused by QoI-resistant isolates may be managed with DMI or MBC fungicides.

A new strategy for RNAseq-based research on plant-fungal interactions: The discrimination of a mixture of pea and *Sclerotinia* ESTs from next generation sequencing without reference genome sequences

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RNAseq is a powerful tool for transcriptome analysis and has been used in many recent studies. However, it has rarely been used to study plant-pathogen

interactions because it is difficult to distinguish plant and fungal ESTs and even virus or viroid contamination, particularly when reference genomes are not available. Here we introduce a strategy for distinguishing plant and fungal ESTs using the TBLASTX program. To verify the feasibility of the strategy, 17,533 *Sclerotinia sclerotiorum* and 18,547 *Pisum sativum* ESTs were downloaded from public databases and pooled. After vector trimming and virus or viroid contamination removal, 35,688 mixed ESTs were used for TBLASTX query of 3 legume genome databases and 7 ascomycete fungal genome databases. By comparing the results from two sets of databases, 90.1% of ESTs were separated successfully into pea ESTs and *Sclerotinia* ESTs, 5.5% had no homology, 4.3% had high similarity to both plant and

fungal genome databases, and just 0.1% were misallocated. Using this technique a set of short 454 sequence reads from pea tissue infected with *S. sclerotiorum* were analyzed. After quality control and adaptor trimming, 128,720 reads with an average read length of >146 nucleotides were assembled into 10,158 contigs by ABYSS program. After discarding 51 contigs with high similarity to virus and viroids, 10,107 contigs were analyzed with TBLASTX. Subsequently, 53.8% of contigs were assigned to pea, 27.4% were assigned to *Sclerotinia*, 5.4% were assigned to both pea and *Sclerotinia* and only 13.4% were unassigned. To validate the results, a set of PCR primers were designed to a random set of ESTs from each assigned group and are currently being used to confirm the TBLASTX EST assignment method.