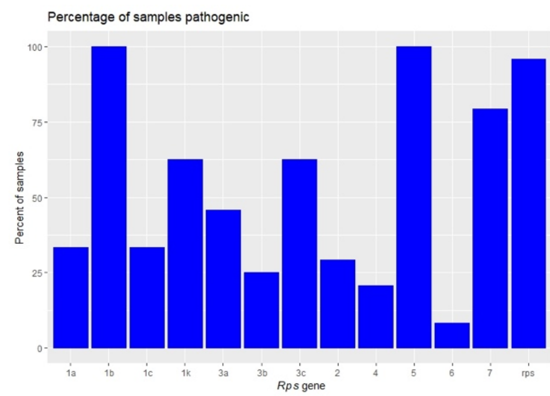
Project Title: Characterization of *Phytophthora sojae* and *Phytophthora sansomeana* populations in the North Central Region AND an Assessment of Management Strategies

Final Report

1. **Recover *P. sojae* from fields within each state and characterize for pathotype and genetic diversity.**

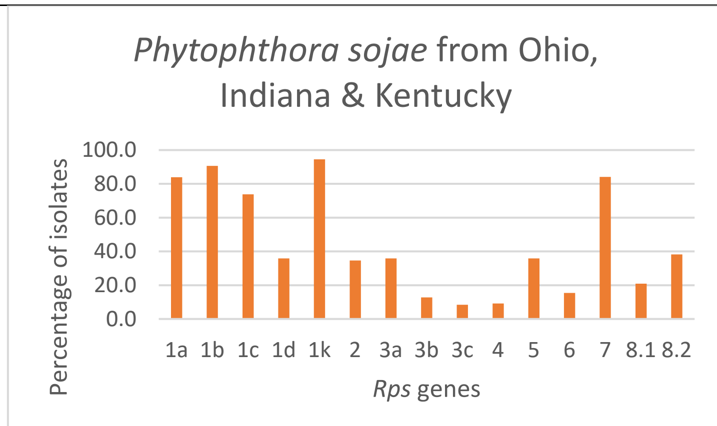
a) Illinois

- IL reported previously that they had completed soil baiting from 50 fields in 26 counties. There were recovered 24 *P. sojae*, 7 *P. sansomeana*, and 148 *Pythium spp.*
- Pathotyping of P. sojae has been results show that *Rps3b, 4* and *6* were the most effective genes, while *Rps1b* and *5* were not effective against any of the collected isolates from IL. The mean complexity of the isolates was 6.0.



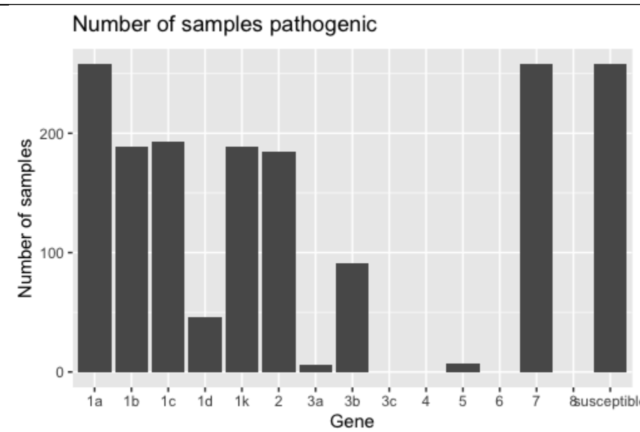
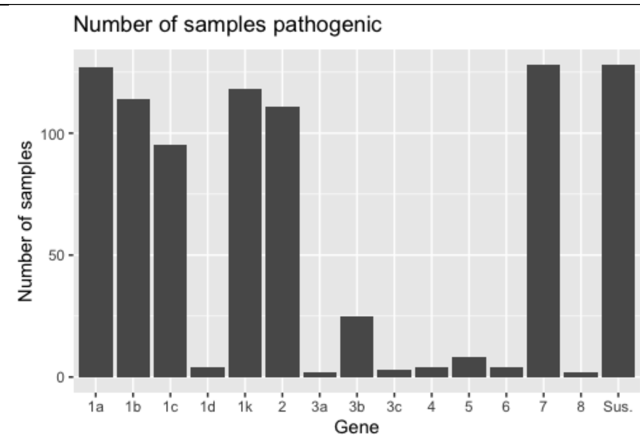
b) Ohio – Indiana – Kentucky

- From the 1,100 soil samples collected from 110 fields in Ohio, Indiana, and Kentucky that 532 isolates of *P. sojae* and 126 isolates of *P. sansomeana*, were recovered through soil baiting.
- Pathotype complexity, the number of *Rps* genes that are not effective ranged from 5.2 to 15.0 among isolates from Indiana, Kentucky, and Ohio. The *Rps* genes *Rps1a*, *Rps1c*, *Rps1k* were ineffective in most locations while *Rps3a* and *Rps6* had some locations where they may be effective.
- Genetically – there were 5 subpopulations identified among the isolates collected from Kentucky, Ohio, and



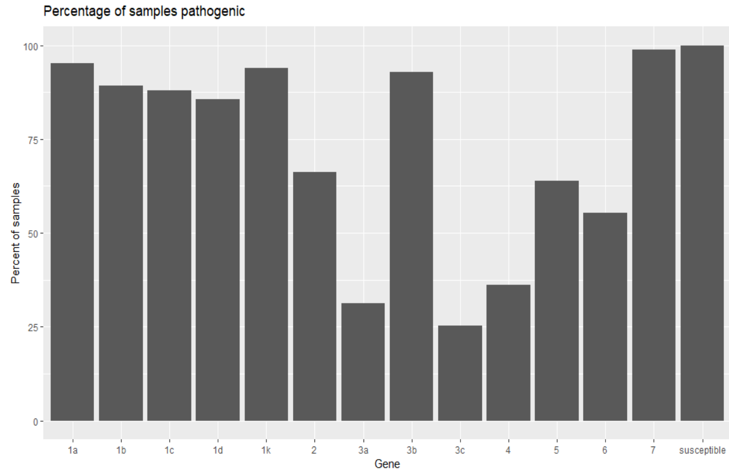
Indiana, with Kentucky’s *P. sojae* population separate from Ohio and Indiana.	

c) Iowa – Nebraska

Response of *P. sojae* isolates from Iowa • 258 isolates of *P. sojae* and 2 isolates of *P. sansomeana* were recovered after soil baiting of 250 soil samples collected from 25 fields in Iowa. • Mean pathotype complexity was 5.5. • Rps genes *Rps1a, Rps1b, Rps1c, and Rps1k* were ineffective and *Rps3c* and *Rps6* were the most effective resistance genes against the isolates collected.	
Response of *P. sojae* from Nebraska • 161 isolates of *P. sojae* collected in Nebraska were pathotyped. • Mean pathotype complexity was 6.3. • Rps genes *Rps1a*, *Rps1b*, *Rps1c*, and *Rps1k* were ineffective, while *Rps3a. Rps3b, Rps3c, Rps6 and Rps8* were effective.	

d) Michigan-

In total 69 fields were sampled from 21 counties within the state of Michigan. Of these 69 fields, 21 were confirmed for *P. sojae* via soil bioassay and isolation. In total, 127 isolates of *P. sojae* and 7 isolates of *P. sansomeana* were recovered from these 21 fields. Eighty-three *P. sojae* isolates were subjected to resistance gene testing via the hypocotyl inoculation technique. Our study has found that genes 3a, 3c and 4 were the most effective at controlling the *P. sojae* population within Michigan.



e) Minnesota

- Completed sampling of 66 fields in 57 counties. 113 isolates of *P. sojae* obtained during baiting. Of these isolates 15 were new pathotypes of *P. sojae*.
- No *P. sansomeana* and 43 *Pythium* isolates were also collected in the 2019 sampling.
- Pathotypes overcoming Rps 1a, 1c, 1k are most common among Minnesota isolates. However, fields with pathotypes overcoming Rps 3 and 6 have been found in this and earlier surveys.

f) South Dakota –

From 155 Phytophthora isolates baited from soil from 27 fields, 145 were *P. sojae* while 10 were confirmed as *P. sansomeana*. Detection of *P. sansomeana* was the first in the state and a first report has been published (in press) in Plant Disease journal. Pathotyping was done for the 145 *P. sojae* isolates and Rps genes Rps1a, 1b, 1c, and 7 were frequently defeated by isolates from a number of fields while Rps 2, 3a, 3b, 3c, 4, 5, and 6 were seldom defeated indicating that the latter genes may be effective against *P. sojae* in SD.

g) North Central Region Summary

To date, 1345 isolates of *P. sojae* have collected along with 142 of *P. sansomeana* and 191 *Pythium* spp. From the isolates of *P. sojae*, 1142 have been pathotyped. The number of *Rps* genes that a specific isolate can cause disease on continues to

increase across the region, with several states reporting isolates that cause disease on all of the *Rps* genes. The implications to this finding are that it will be difficult if not impossible to rotate genes. These populations of P. sojae, once they are adapted are maintaining virulence in the field. There are regional differences, similar to what was reported earlier but *Rps1a, Rps1c*, and *Rps1k* were largely ineffective across the region.

2. Evaluate the new sources of resistance to these regional populations.

- Due to poor harvest in 2018 and spring planting challenges in 2019, this remains a challenge to provide everyone enough seed to evaluate these new sources.
- Minnesota – RpsUN1 & RpsUN2 have been incorporated into Minnesota adapted lines that are being increased for further testing

3. **Recover *P. sansomeana* from fields within each state and characterize for host range, genetic diversity.**

- IL - We obtained seven *P. sansomeana* isolates from soils in Illinois, we are evaluating their aggressiveness to soybeans.
- IA – Two isolates of *P. sansomeana* were recovered in Iowa. Both came from the same field.
- MI - 18 isolates of *P. sansomeana* from various states within the U.S. have been tested against 6 potential hosts of agronomic importance (corn, soybean, wheat, winter rye, oats and red kidney beans) in a growth chamber assay. Preliminary results show legumes to be susceptible hosts, while monocot candidates exhibit little to no symptoms, with the pathogen still being able to be isolated from root tissue. The role these plants play as potential reservoir or non-symptomatic hosts is currently being investigated via a *P. sansomeana* species specific qPCR assay (Rojas et al., 2017) to determine the level of colonization of root tissue.

4. Establish sensitivities (EC_{50}) values for *P. sojae* and *P. sansomeana* isolates recovered from fields towards the new active ingredients ethaboxam, strobilurin, and oxathiapiprolin fungicides.

- IL: Nine isolates of *P. sojae* and seven of *P. sansomeana* were sensitive to ethaboxam, mefenoxam, and metalaxyl at 1 µg mΓ ^{l}. All isolates were sensitive to azoxystrobin at 2 µg mΓ ^{l}, except for one isolate of *P. sansomeana* that was less sensitive (EC_{50} of 3.5).
- MI- Currently, mefenoxam, ethaboxam, oxathiapiprolin and pyraclostrobin EC50 values are being determined using a high throughput fungicide. sensitivity assay developed in the Chilvers lab (Noel et al., 2019). Fungicide sensitivity testing is nearly complete, results will then be written up and submitted for publication. Thus far there have been no isolates of P. sojae identified which are insensitive to any of the four chemistries tested.

- MI - Phytophthora EC50s to four seed treatments is being evaluated for *P. sansomeana*. Preliminary data suggests that there is no rise in *Phytophthora sansomena* resistance to Mefenoxam, Ethaboxam and Oxathiapiprolin.
- MN - EC50 study: Three fungicides will be evaluated in the initial, mefanoxam, metalaxyl, and ethaboxam. Strobilurins, Azoxystrobin, Trifloxystrobin, and Pyriclostrobin, will be evaluated if time permits. However, we are delaying evaluation of these strobilurins because they were previously test against the same group of isolates. The EC50's observed were several orders of magnitude higher than the oomyceticides.
- SD – testing has been completed for ethaboxam (EC50 range from 1 to 25 µg), oxathiapiprolin (0.5 to 50 µg) and strobilurins, . All isolates were sensitive to ethaboxam and oxathiopiprolin.

5. Comparison of new seed treatments on varieties with different resistance packages (*Rps* gene(s)/partial resistance) in field trials.

- NE – published results from 2017 & 2018 comparing the efficacy of a fungicide seed treatment on cultivars with different levels of resistance. Seed treatments increased emergence in 4 of 5 environments. Canopy coverage estimates were useful tools in this study. Combining host resistance with seed treatments were beneficial overall. They misnamed the partial resistance in these cultivars with company terminology –
- IL and IA conducted multilocation field experiments of varieties with various *Rps* genes and treated with Intego suite. Data analysis is being fine-tuned and a manuscript draft is ready. Expect to submit by June.
- IA conducted a trial in 2018. Stand count differed among varieties ($P<0.01$) but not between treated and non-treated seed of each variety. No treatment effect of yield was detected ($P=0.32$).

- Multi-state field trial sampling has been completed for the 2019 season (IN, MN and MI). Yield will be recorded when the plots are harvested
  - **MN -** Variety/Seed Treatment study planted in early June *followed by regular weekly irrigation or rain since planting.* Plots inoculated with multiple *P. sojae* pathotypes. Collected field data, stand counts, emergence, diseased plants, etc. on Variety/Seed Treatment study. The data collected and forwarded to Austin. All weights, measures,stand counts for the three developmental stages are completed- Vc, V2+ and R4 and final yields harvested We observed no significant effect of variety or seed treatment on yield. A complete results and data analysis will be reported in combination with those from MI and IN.
  - IN - A trial was planted at the Pinney Purdue Agricultural Center, in Porter County Indiana on June 6, 2019. The trial consisted of four varieties with three different seed treatments (untreated, base seed treatment and Intego Suite seed treatment), all plots were inoculated with *Phytophthora sojae* in-furrow at planting. Irrigation was applied weekly at 1 in.

- MI - Multi-state field trial sampling has been completed for the 2019 season (IN, MN and MI). No significant differences in yield observed 2019. qPCR is underway on V2 root samples to quantify *P. sojae* in each treatment. Writing will begin soon on the field portion of the project

6. Evaluation of potential herbicide interactions with the development seed rot and stand loss.

IA - Field trials to test the effect of PPO injury of the susceptibility of soybean to seedling disease were established at the ISU Southeast (SERF), Ag Engineering and Agronomy (AEA), and Horticulture research farms in 2018. All trials were inoculated in 2018, but not 2019. In 2018, the trial at the Horticultural farm was irrigated as seedlings emerged. No irrigation was done at the AEA and SERF. No symptoms of PPO damage or seedling disease were observed in both years.

OH – only one of two field trials was established to compare the susceptibility of soybean seedlings to injury from different PPO herbicides applied after planting. Poor conditions contributed more to injury in 2019- experiment was dropped.

IN- Two field trials were established, 1) Pinney Purdue Agricultural Center (PPAC), in Porter County Indiana planted on June 6, 2019, and 2) Purdue Agronomy Research and Education Center (ACRE) on June 4, 2019. Each trial was planted with AG27X8 variety and inoculated with *Phytophthora sojae* in-furrow at planting. Irrigation was applied weekly at 1 in. at PPAC, but not ACRE. Pre-emergence fungicide treatments were applied on June 7 at ACRE and June 8 at PPAC. The treatments consisted of 1. Non-treated control; 2. Spartan (sulfentrazone) 10 fl oz/A; 3. Valor (flumioxazin) 2.5 oz/A; 4. Sencor (metribuzin) 2/3 lbs/A; 5. Sharpen (saflufenacil) 1 fl oz/A; 6. Classic (chloriumuron) 2.5 oz/A.

- Data collection has been completed, which included stand counts (VC, VC+14, VC+28), counts of plants damping off, vigor and PPO injury incidence was recorded, Canepeo images, and final disease assessment at R6
- Root samples were collected and roots were plated for pathogen identification.

7. Development of Crop Protection Network Outputs on the Management of *Phytophthora* spp. that infect soybean in the North Central and Ontario regions.

- *Draft is in progress all states will be cooperating- now that pathotyping is complete*

Extra activities not directly in the original objectives

- IL - The ITS region was sequenced for 65 of our *Pythium* isolates and identified eight species. Most of our isolates were *Pythium ultimum* var. *ultimun*.

- IL- evaluated fungicide sensitivity of 11 *Pythium* isolates. All isolates were sensitive to metalaxyl and mefenoxam with EC_{50} ranging from 0.033 to 1.4 µg ml^{-1}. Two isolates of *P. vexans* (18 %) were insensitive to the highest dose of azoxystrobin (10 µg ml^{-1}). Three *P. aphanidermatum* isolates (27 %) were insensitive to the highest dose of ethaboxam (100 µg ml^{-1}). A poster was presented about these results at the Plant Health 2019 meeting in Cleveland.
- IL - In collaboration with N. Kleczewski, we evaluated the aggressiveness of 65 of our Pythium isolates against soybean and corn and evaluating the aggressiveness of our P. sojae isolates against soybean.
- MI – McCoy, Noel, Sparks, Chilvers produced an R package to perform pathotype data analysis. A resource announcement was published in Molecular Plant-Microbe Interactions October 2019. **It was one of MPMI's top 10 most downloaded articles of 2019.**

Presentations/Abstracts

Cerritos-Garcia, D., Huang, S-Y., Kleczewski, N. M., and Mideros, S. 2019. Diversity and fungicide sensitivity of Phytophthora pathogens of soybean in Illinois. Poster. Presented at Annual meeting of American Phytopathological Meeting in Cleveland, OH

Cerritos-Garcia, D., Kangas, C., Kleczewski, N. M., and Mideros Mora, S. X. 2019. Characterizing Pythium associated with Illinois soybean fields. Poster. Presented at Annual meeting of American Phytopathological Meeting in Cleveland, OH.

Hebb, L.M., Dorrance, A.E., Bradley, C., Wise, K., Telenko, D. 2019. Phytophthora sojae pathotype variability across Ohio, Indiana, and Kentucky. Poster. Presented at Annual Meeting of American Phytopathological Meeting in Cleveland, OH

Hebb, L.M., Dorrance, A.E., Bradley, C.A., Telenko, D., and Wise, K. 2019. Population structure and genetic diversity of Phytophthora sojae across Ohio and Indiana. Poster. Presented at Oomycete Molecular Genomics Network Annual Meeting.

McCoy, A., Telenko, D., Dorrance, A.E. and M. Chilvers. 2019. Variety genetics and oomycide seed treatments effect on stand, health, and yield of soybeans under *Phytophthora sojae* disease pressure. Poster. Presented at Annual Meeting of American Phytopathological Meeting in Cleveland, OH

Manuscripts- to date, many are in progress

Garnica, V.C. and Giesler, L.J. 2019. Soybean canopy coverage, population, and yield responses to seed treatment and cultivar resistance to *Phytophthora sojae* in Nebraska. Plant Health Progress 20:229-237.

McCoy, A. G., Noel, Z., Sparks, A. H., & Chilvers, M. (2019). Hagis, an R Package resource for pathotype analysis of phytophthora sojae populations causing stern and root rot of

soybean. *Molecular Plant-Microbe Interactions*, *32*(12), 1574–1576. https://doi.org/10.1094/MPMI-07-19-0180-A

Tande, C.A., Dorrance, A.E., Schwazrock, D., Mahecha, E., and Byamukama, E. 2020. First report of *Phytophthora sansomeana* causing root rot of soybean in South Dakota. Plant Dis.. *First Look online*

Theses:
Arneson, N.J. 2019. Effect of soil-applied protoporphyrinogen oxidase inhibitor herbicides on soybean seedling disease. Masters Thesis. University of Nebraska-Lincoln, Lincoln, NE.