



## North Central Soybean Research Program

### **Characterization of *Phytophthora sojae* and *Phytophthora sansomeana* populations in the North Central Region and an Assessment of Management Strategies**

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#### **Progress Report April 2019**

*Phytophthora sojae* is present in many soybean fields across the north-central region of the U.S. and Ontario, Canada and had been managed very successfully with the deployment of single resistance Rps genes (Rps1a, Rps1c, Rps1k, Rps3a, and Rps6) as well as partial resistance (field resistance or tolerance). However, there are an increasing number of reports where varieties are sold that have Rps genes that are no longer effective towards the regional population or lack sufficient levels of partial resistance. The consequence is stand loss from damping-off or the development of stem rot throughout the season which results in lower yields and added weed pressure.

The most recent survey of *P. sojae* pathotypes (isoalates differing in pathogenicity) was completed during 2012/2013. In that study, two key findings indicate that the ability to recycle Rps genes is highly unlikely:

- 1) a greater number of regions have a higher proportion of isolates with virulence to key Rps genes, such that the resistance gene will no longer be effective
- 2) *Phytophthora* isolates across the region continue to increase in complexity.

New Rps genes have been identified but their effectiveness is still highly questionable, as only 5 of the original 15 Rps genes were effective and incorporated into varieties.

A second *Phytophthora*, *P. sansomeana*, also appears to be contributing to seed and seedling damping-off of soybean at greater incidence than previously thought. This species will be examined for host range, resistance in current soybean cultivars, and efficacy of seed treatment fungicides.

## Project Objectives

1. Recover *Phytophthora sojae* from fields within each state and characterize for pathotype and genetic diversity.
2. Evaluate the new sources of resistance to these regional populations.
3. Recover *Phytophthora sansomeana* from fields within each state and characterize for host range, genetic diversity.
4. Establish sensitivities (EC50) values for *P. sojae* and *P. sansomeana* isolates recovered from fields towards the new active ingredients ethaboxam, strobilurin, and oxathiapiprolin fungicides.
5. Comparison of new seed treatments on varieties with different resistance packages (Rps gene(s)/partial resistance) in field trials.
6. Evaluation of potential herbicide interactions with the development seed rot and stand loss.
7. Development of Crop Protection Network Outputs on the Management of *Phytophthora* spp. that infect soybean in the North Central and Ontario regions.

## Research progress April 2019

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

- Isolate collection of >2000 isolates (200 isolates per state)
- Improved understanding of diversity of pathotypes across region will inform of areas where Rps genes can and cannot be deployed

Ohio: Pathotyping results on 206 isolates of *P. sojae* show that >90% of isolates were able to overcome Rps1a, Rps1b, Rps1k and Rps7. Rpsa3b, Rps3c, Rps4 and Rps6 were the most effective.

Indiana: 122 isolates of *P. sojae* have been pathotyped from the state of Indiana, and overall Rps genes were more effective in Indiana than against isolates from Ohio. Rps3c, Rps4 and Rps6 were the most effective, where <5% of isolates were able to overcome these Rps genes. Rps1b and Rps1k were highly ineffective against Indiana isolates.

Kentucky: 88 isolates have been pathotyped from the state of Kentucky, and results were similar to Indiana.

From the pathotyping of 416 isolates of *P. sojae* in Ohio, Indiana and Kentucky, we see some Rps genes with similar degrees of effectiveness across all areas, but also variation in the effectiveness of other Rps genes. These results and the intensity of sampling provides valuable and current information on what Rps genes should be deployed where. Testing of genetic diversity of 192 isolates of *P. sojae* from Ohio, Indiana and Kentucky is underway. The results of this testing will provide information on the presence of subpopulation structures of *P. sojae*

across these states.

Michigan – Rps genes 3a, 3c and 4 were the most effective

Illinois - Twenty-four isolates of *P. sojae* are being pathotyped. Preliminary results show that 20% and 60% of Illinois *P. sojae* isolates are able to overcome Rps1c or Rps1k, respectively.

#### *Additional Development* –

The first improvement in *P. sojae* pathotype data analysis in two decades is forthcoming: Austin McCoy and Zachary Noel at Michigan State University have developed R code which can perform the same analyses of virulence data as the HaGiS excel spreadsheet. This code has been accessioned with a DOI and is freely available at:

<https://github.com/AGmccoy/Phytophthora-sojae-Pathotype-analysis>. Adam Sparks at the University of Southern Queensland is currently working with Austin and Zachary to develop this code into a more user friendly and freely available R package for current and future *P. sojae* pathotype studies. The advantages of the forthcoming R package are easily reproducible data and elimination of potential user error of data input into the HaGiS sheet.

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

- Identify Rps genes that are effective for each of collaborating states
- Improved deployment of soybean varieties with Rps genes across the region will result in fewer losses due to Phytophthora root rot

During 2018, many lines with sources of these genes were increased and are still in the field. Currently evaluating how to increase these lines for further evaluations and testing.

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

- Isolate collection of >600 isolates (50 isolates per state)
- Improved understanding of phenotypic and genetic diversity within this species
- Improved knowledge of distribution and prevalence of the pathogen across the region
- Knowledge of effectiveness of Rps genes against pathogen

Eighteen 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.

An additional 114 isolates of *P. sansomeana* were recovered from soils collected in Ohio, Indiana and Kentucky. Kentucky yielded the fewest isolates, with 9 *P. sansomeana* isolates recovered, while Indiana isolates of *P. sansomeana* numbered 29. In the state of Ohio, 76 *P. sansomeana* isolates were recovered.

Inoculation of the same differential lines used for pathotyping of *P. sojae* isolates showed some seedling death, but not a consistent resistant/susceptible response. Symptoms were more commonly a general yellowing and wilting of plant tissues. This provides some preliminary data for better characterization of effectiveness of Rps genes against this pathogen. Additionally, the prevalence of this pathogen across different regions is better understood from the level of recovery from soil samples in Ohio, Indiana and Kentucky. In Ohio, field trials planned for this summer will directly test the ability of *P. sansomeana* to cause disease on susceptible soybean cultivars, and measure any yield reductions in comparison to both non-inoculated and *P. sojae*-inoculated soybean.

4. Establish sensitivities (EC50) values for *P. sojae* and *P. sansomeana* isolates recovered from fields towards the new active ingredients ethaboxam, strobilurin, and oxathiapiprolin fungicides.

MI, IL, SD

- Establish baseline levels of fungicide sensitivity to ethaboxam and oxathiapiprolin before these active ingredients are widely used.
- Monitor sensitivity to strobilurins and detect reduced sensitivities that may have occurred in past few years based on published data (Broders et al 2007; Matthiesen et al 2016; Rojas et al, submitted) due to widespread use in seed treatments containing strobilurins.
- Information on placement of active ingredients across the region

Currently a subset of isolates are being tested to determine the optimum concentration of SHAM (salicyl-hydroxamic acid) to use when determining EC50 concentrations of the strobilurin fungicide pyraclostrobin. Once this is done, mefenoxam, ethaboxam, oxathiapiprolin and pyraclostrobin EC50 values will be determined using a high throughput fungicide sensitivity assay developed in the Chilvers lab (Noel et al., accepted 2019).

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

In the 2018 field season, nine varieties of soybean, each containing a different *Phytophthora*

resistance package, were planted in an inoculated field trial site. Each variety was treated with 3 different seed treatments; 1.) non-treated, 2.) Ipconazole and Clothianidine and 3.) Ipconazole, Clothianidine, mefenoxam and ethaboxam. Plant stand, dry weights and qPCR were performed at 3 separate time points throughout the growing season (VC, V2 and R4) as well as yield.

All varieties exhibited significantly increased stand establishment with the use of the Ipconazole, Clothianidine mefenoxam and ethaboxam seed treatment. Three varieties had significantly increased stand with the use of the Ipconazole, Clothianidine mefenoxam and ethaboxam seed treatment compared to the base or non-treated seed. Further testing with qPCR and partial resistance testing for the 2018 field season is ongoing.

The 2019 field season will be much the same. All the seed is ordered or has arrived and will be treated as in 2018. Indiana will also be participating in the 2019 field season but will be planting 4 varieties instead of the 9 that Michigan will be. Varieties will be acquired by Michigan State University, treated, and then distributed to Indiana so that both states may have the same planted varieties.

In 2017 and 2018, a total of 6 field experiments were conducted in Nebraska and Iowa. We selected commercial soybean cultivars carrying either Rps1k or Rps1c genes with distinct PSRR tolerance scores to represent moderately resistant [MR] or moderately susceptible [MS] classes, based on company-provided PSRR susceptibility scores. For the seed treatment component, a commercial formulation containing ethaboxam, metalaxyl, ipconazole, and clothianidim (Intego Suite Soybeans, 3.37 fl oz/100 lbs, Valent, USA) was applied to seeds prior to planting and a non-treated control was added for comparison.

From these trials, we recorded data on plant population, canopy coverage, number of plants with *P. sojae* stem lesions, and yield. Preliminary analysis indicated significant differences on responses collected as result of seed treatment use and cultivar selection towards PSRR management. In summary, seed treatment significantly increased seedling emergence, canopy coverage, and yield in at least 3 locations in 2018 but none in 2017.

Management programs using MR cultivars had a positive yield effect compared to MS cultivars, particularly in locations where moderate damping-off and subsequent *P. sojae* stem lesions developed. There was no strong evidence of yield difference between cultivars carrying Rps genes, except in one location, where Rps1c cultivars yielded more than Rps1k cultivars. Correlation analysis were also conducted to determine the relationship between responses collected.

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

- Field trials will be established at least 2 locations per state during 2017 and 2018.
- Effect of different resistance packages on soybean disease and midseason PRR.
- Detect interactions between pre-emergence herbicides and watermold pathogens that increase risk of stand loss due to seedling disease
- Economic benefits/costs for the each management tactics.

In 2017 and 2018, a total of 6 trials were conducted in the eastern portion of Nebraska. Fields selected were relatively poorly drained and had history of stand establishment issues. Five single active ingredient PRE herbicides were applied shortly after planting. Plots were maintained weed-free with one or two post-emergence applications of glyphosate and hand-weeding as needed throughout the season.

At each location, we collected plant population, root rot ratings at VC-V1 growth stage, fresh root and shoot weight, herbicide injury ratings, plant height, and yield. Root rot ratings collected on a 0-10 scale were converted into a disease severity index (DSI). We also performed systematic isolations of the roots scored at each location, in case potential responses were associated with the prevalence of primary seedling pathogens (Pythium, Fusarium, Phytophthora, and Rhizoctonia species).

For most of the components evaluated, our results are not supportive that PRE herbicides consistently affect seedling disease incidence under field conditions. Chlorimuron-ethyl, metribuzin, saflufenacil, sulfentrazone, and flumioxazin applied PRE at labelled rates did not significantly impact DSI or soybean stand when compared to non-treated control. In one environment, PPO-inhibiting herbicides (saflufenacil, sulfentrazone, and flumioxazin) seemed to have a detrimental effect on root health status (DSI) when contrasted to non-PPO-inhibiting herbicides (chlorimuron-ethyl and metribuzin) but this response was not consistent with other parameters evaluated (yield, stand, fresh shoot and root weight). Also, there were no significant yield differences between PRE herbicides and the no herbicide control and phytotoxicity was low throughout the study.

Overall, our results indicate no clear association between PRE herbicides and seedling diseases under field conditions which supports the use of these herbicides as part of an integrated weed management program in late-planted soybeans. More field studies will be conducted in 2019.

In the Midwest, as early-plantings become more common, the incidence of seedling diseases favored by cool and moist soil conditions (e.g. Pythium damping-off) may increase. Additional

studies would be necessary to determine potential synergism between PRE herbicides and seedling diseases in these scenarios.

**Why this is important**

- Improved management recommendations for soybean farmers for *P. sojae* and *P. sansomeana*
- More importantly, information for companies on which of the Rps genes should be deployed in the region
- Baseline values of new watermold fungicides to know what will be effective in region
- Key information to limit losses from herbicides