Final Report - Enhancing Phenotyping for Resistance to Sudden Death Syndrome (SDS) of Soybean4
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Objective 1: To determine the relative importance of resistance to root infection compared with resistance to foliar symptom expression. The goals of this objective are to:

- **Determine the effectiveness of root rot or foliar symptom resistance with increasing inoculum density.**
- **Characterize the yield response of soybean varieties expressing either resistance as resistance to root rot 30 days after planting or resistance to foliar symptom expression during early vegetative growth stages and during late reproductive development.**

Three preliminary experiments were conducted:

1) for this study. Initially we worked to determine the best method of growing the soybean plants in order to recover roots for estimation of root rot severity.
2) The subsequent two trials (Trial 1 and 2) included improvements in the pot type used and additional treatments (eg sorghum).

Trial 1:
Two treatments (control and treated with *Fusarium virguliforme*) each replicated five times for three varieties; Minsoy, McCall and Noir1 were established in a growth chamber operated at 21C with 14 hour daylength. Inoculum was evenly distributed through the medium; each pot had four seedlings which sampled at four growth stages Ve, Vc V1, and V2, to determine root infection.
Root length, root dry weight, and root necrosis and development of both tap and secondary roots.
Observations

- Ve, few secondary roots present, no necrosis and untreated plants had greater root length and dry root weights than treated plants.
- VC, on average, inoculated plants had more secondary roots and greater dry root weight than uninoculated which can be referred to as root proliferation.
- V1 foliar symptoms of SDS present. In contrast with VC, V1 the uninoculated plants had more secondary roots and greater root weight than the inoculated, Root necrosis symptoms greatest on inoculated McCall and foliar symptoms greatest on inoculated Minsoy.
- V2, inoculated Noir 1 showed greater tap root necrosis than inoculated Minsoy and Noir. Inoculated Minsoy had more severe foliar and secondary root necrosis than either inoculated Minsoy or Noir.
- Due to the difficulty in untangling the roots in the jumbo pots, further studies will be conducted with only one plant per pot. In addition, a third treatment which will be sorghum only added.

Trial 2:

Three varieties; Minsoy, McCall and Noir1 organized in three treatments (uninoculated, inoculated with uninfested sorghum and inoculated with *Fusarium virguliforme infested sorghum*) planted in three replications were maintained in a growth chamber at 21C with 14 hour days. Single plants were established in conetainers, each conetainer representing a single growth stage; either Ve, Vc, V1 or V2. Inoculant, both infested and uninfested, was evenly distributed through throughout the planting medium.
The root variables, root length, dry root weight, root necrosis for both tap and secondary roots were recorded at four growth stages, Ve, Vc, V1 or V2.

Observations

- Ve, the untreated plants had higher dry root weights and root length than those receiving uninfested sorghum. Inoculated plants had smaller dry root weights and root lengths than either control treatment.
- Vc, Noir1 with added uninfested sorghum had longer root length than either the sorghum-free treatment or the infested, inoculated treatment.
- V1, inoculated Minsoy had shorter roots, more severe tap and secondary root necrosis and foliar symptoms but than either McCall or Noir1. Foliar symptoms of SDS first appeared at V1 on Minsoy.
- V2, inoculated Minsoy had shorter roots, more severe tap and secondary root necrosis than either McCall or Noir1. Foliar symptoms severity increased until V1.

This experiment was conducted during May, June, July, August, and September 2018. Twenty-five soybean varieties were planted in the greenhouse. The soybean varieties had been characterized in previous experiments as either resistant or susceptible to leaf scorch or to root rot caused by SDS based on previous results obtained in field and greenhouse trials. Varieties included Ancestral Lines of the UMN soybean breeding program and RIL crosses of MinSoy and Noir 1. Maturity groups 00-2 were represented.
Each variety was planted in three treatments: 1) soil infested with *Fusarium virguliforme*, 2) untreated soil and 3) soil with autoclaved uninfested red sorghum. The complete variety and treatment planting was replicated 3 times on 25 varieties in "tall" 2 gallon 6" x 16" "tree pots". The "tall" pots allowed for vertical tap root growth. Days to takedown were expected to be appx 100-120 DAP taking each variety to yield at physiological maturity.

Pots were treated with Osmocote 14-14-14 and inoculated with NCharge rhizobia. A soil applied insecticide, Marathon, was applied to control greenhouse insects. Inoculum of *Fusarium virguliforme* was applied at a rate of 50 ml of infested red sorghum with a concentration rate of 5.0 x 10^6 spores/ml per pot in the inoculated, infested treatment.

Two seeds were planted in each pot and one seedling culled at Vc leaving a single plant per pot. Automatic atering system installed to support 225 two gallon pots for appx 120 days. Water was applied daily at a rate of 32 ounces/pot over 15 minutes via drip line (Netafim). No light supplementation was provided because plant growth took place during the growing season.

Data collected:

- Stand Count
- SDS foliar necrosis scores taken twice/week during June, July and August
- Plant fresh weight taken at harvest.
- Dry seed weight/yield taken after dry down and hand threshing.

Further data analysis is underway.

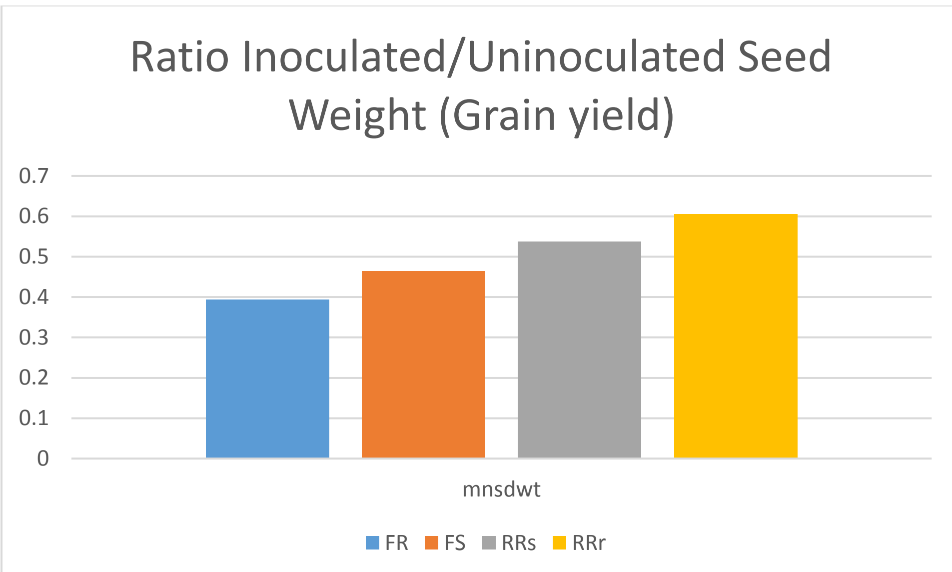

Fig. 1. Ratio of grain yield when inoculated grain yield is compared to uninoculated grain yield (inoculated yield/uninoculated yield) of four classes of soybean varieties characterized as either resistant or susceptible to root rot or foliar scorch. FR = Foliar resistant, FS = Foliar susceptible, RRs = Root rot resistant, RRr = Root rot susceptible.

Results:

Resistance to root rot resulted in greater final yield and lessened yield loss to Fv infection when compared to yield loss of varieties considered to be resistant to foliar symptom expression (leaf scorch).

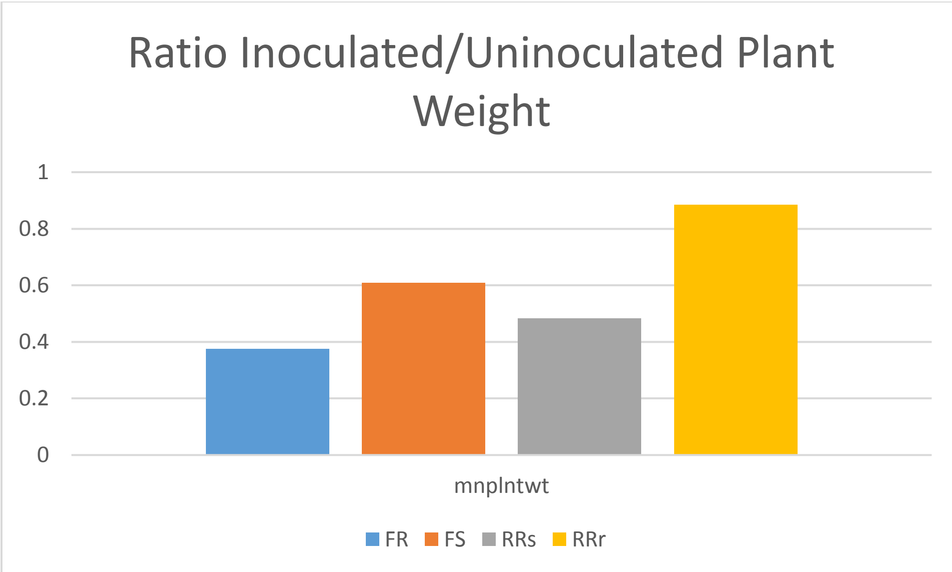

Fig. 2. Ratio of plant dry matter when inoculated dry matter is compared to uninoculated dry matter (inoculated dry matter /uninoculated dry matter) of four classes of soybean varieties characterized as either resistant or susceptible to root rot or foliar scorch. FR = Foliar resistant, FS = Foliar susceptible, RRs = Root rot resistant, RRr = Root rot susceptible.

Results:

Resistance to root rot resulted in greater greater dry matter production and lessened effect of Fv infection when compared to dry matter loss of varieties considered to be resistant to foliar symptom expression (leaf scorch) (Fig. 1 and 2).

Discussion:

Current phenotyping relies on foliar symptom severity as an indication of variety resistance or susceptibility. Evaluation of variety resistance to root rot might be more rewarding in maintaining soybean yield in the presence of SDS.

Objective 2: To characterize experimental requirements, light intensity and growth media, that facilitate repeatable disease assessments. The goals of the objective are to:

- **Identify inoculum substrates that consistently support SDS infection and symptom development.**

SDS Vermiculite as substrate trials (Lab)

Studies were conducted using vermiculite as the substrate and carrier for inoculum of SDS pathogen, *Fusarium virguliforme* and for comparison with FV infested sorghum which is currently used for inoculation. Our experience has been that sorghum can cause confounding visible symptoms similar to those of root rot caused by FV including root browning, reduced plant biomass, and sometimes root pruning.

We determined that vermiculite impregnated with a variety of nutrient sources; soybean broth, potato dextrose broth, and cornmeal broth, will support sporulation by *F. virguliforme* without the confounding effects of the sorghum inoculum substrate. Vermiculite impregnated with soybean broth provided the best growth of the pathogen after 30 days.

A study was also conducted using a plant based “bioplastic” as a carrier for *Fusarium virguliforme*. Results were compared with the vermiculite, sorghum, and cornmeal substrates.

All results were evaluated to determine which method provides the most consist infestation (inoculation) results.

Results: Vermiculite impregnated with a nutrient broth provided the most consistent results. However, we have continued to use “white” coated sorghum for inoculation in our experiments because of the labor involved in producing the alternative inoculants.

- Determine light intensity necessary for the development of foliar symptoms in controlled environments, either greenhouse or growth chamber.

0, 40, 70 % shade

This experiment was conducted twice during November and December 2018 and January and February 2019. During November and December 2018 six soybean varieties were planted in one-liter conetainers in 6 replications. There were two inoculation treatments: treated with sorghum infested with *Fusarium virguliforme* and an untreated treatment. The concentration of the inoculum was 4.2 x 10^5 spore/ ml.
Three environments replicated in six replications include:

- 0% shade
- 40% shade
- 70% shade

Watering was provided daily through an automatic watering system, 350 ml over 5 minutes per conetainer. Water quantity was adjusted regularly to maintain soil moisture at field capacity. Supplemental light was provided via Na Halide lights for 14 hours/day. Light and temperature were monitored via data logger in each environment in each replication.
Soil fertility was maintained with Osmocote was added at recommended rates to the plants.
This experiment was completed 30 DAP with disease rating and plant harvest in mid-December.
Variables observed included root necrosis severity, foliar symptom development, and

accumulated foliar symptom.

0 and 20 % shade

Six soybean varieties were planted in one-liter conetainers in 6 replications. There were two treatments: treated with *Fusarium virguliforme* at 4.2 x 10^5 spore/ ml. and untreated.
Two light intensities are obtained with use of shade cloth:

- 0% shade
- 20% shade

Supplemental light provided by Na Halide lights for 14 hours/day. Light and temperature monitored by data logger at each light intensity in each replication.
The trial was planted and will be harvested 30 DAP.

Results:

Variety evaluations conducted at shade qualities of 0, 40, or 70% resulted in different variety susceptibility rankings when EITHER foliar symptom or root rot severity is evaluated (Fig. 3 and 4). Experiments conducted at 0 and 20% shading yield extremely variable and inconsistent results probably because of the experiments were conducted during periods of very low light intensity.

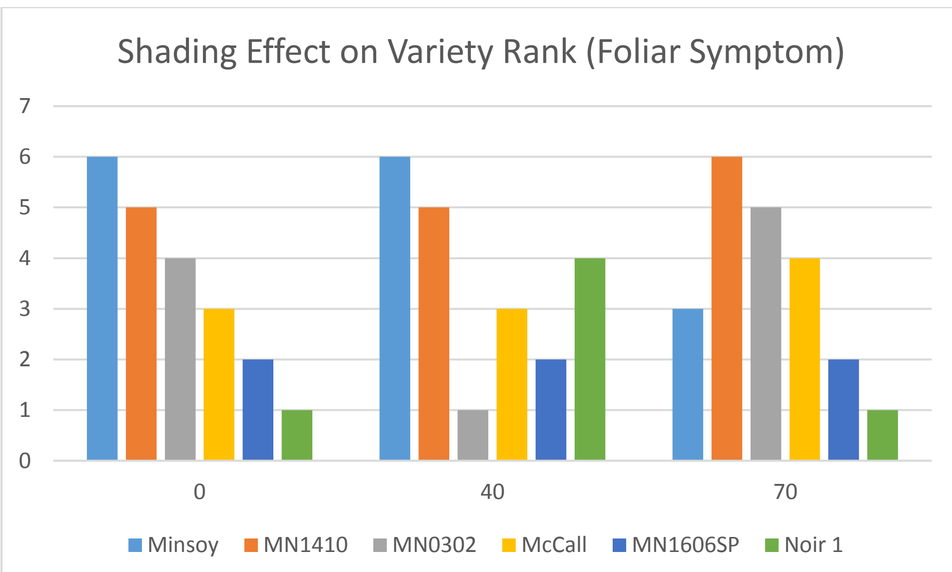

Fig. 3. Shade effect on ranking of varieties (1 is least, 6 is most severe) on basis of foliar symptoms caused Fv infection.

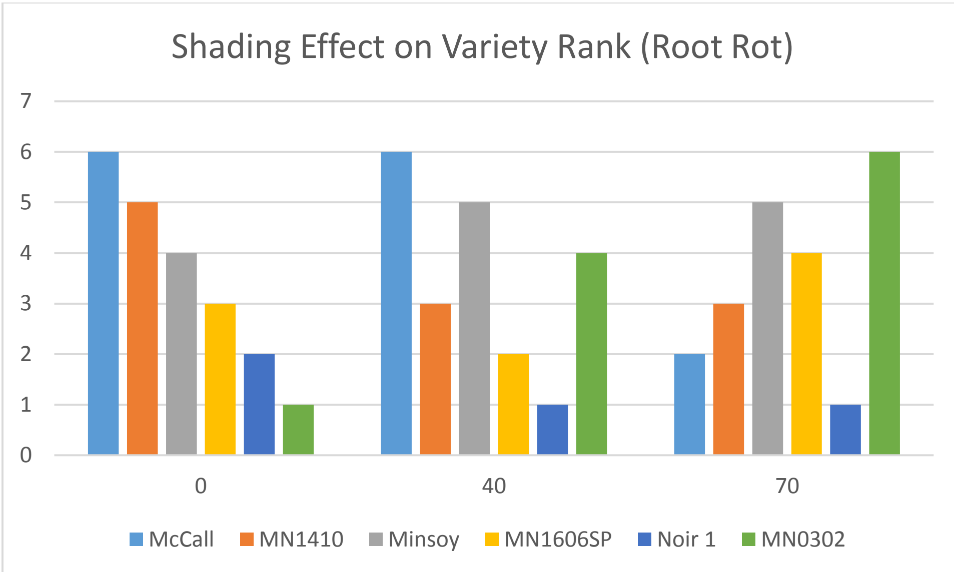

Fig. 4. Shade effect on ranking of varieties (1 is least, 6 is most severe) on basis of root rot symptoms caused Fv infection.

Conclusions:

The results obtained from experiments conducted at 0, 40, and 70% shading indicates that light intensity can profoundly effect the results of variety evaluations conducted in greenhouses under varying light conditions, particularly at light intensities experience during different times in the growing season.

Objective 3: To identify markers for resistance that are less sensitive to the effect of environmental conditions. The goal of this objective is to:

- **Identify objective, quantifiable proxy markers for plant resistance such as biochemical, hyperspectral reflectance, or molecular markers, that enable rapid phenotyping of the response of soybean varieties to Fv infection.**
  Determine if symptoms evaluated with proxy methods such as hyperspectral reflectance, fungal DNA concentration, or toxin concentration correlate with results obtained by visual assessment or physical measurement

Twenty varieties specially selected for their resistance or susceptibility to SDS foliar symptoms and root necrosis were planted at three plants to a microplot in five replications at Rosemount Outreach Center in mid-May. The inoculation treatments included an 1) uninoculated (soil-only), 2) inoculated with autoclaved, uninfested sorghum, and 3) inoculated with FV infested sorghum treatment. The varieties were planted in paired side-by-side planting of inoculation for each variety. Varieties were randomized within each replication.

Observations conducted throughout the growing season:

Foliar symptom and stand count recorded for analysis (correlation) and comparison with hyperspectral reflectance values.
Hyperspectral reflectance of three leaves in each plot was recorded weekly throughout the growing system.
Plots were harvested in October for use as increases of seed. This will provide freshness of seed and purity of variety identity.

Results:

Data recorded during the growing season are currently being analyzed.

SDS Root Infection Progression (growth chamber)
One trial was begun for this study on 26 January.
Root rot symptom development:
Two treatments (control and treated with *Fusarium virguliforme*) each replicated five times for three varieties; Minsoy, McCall and Noir1 were setup in a growth chamber on 15 February. The root length, dry root weight, root necrosis scores for both tap and secondary roots will be taken at four stages.

- At Ve,
- At VC,
- At V1 through V2 until foliar expression is complete and defoliation occurs.

Root infection damage will be evaluated: root length, dry root weight, root necrosis for both tap and secondary roots are taken at all four growth stages.

Hyperspectral Reflectance

Simultaneously with the physical weight and visual symptom observation hyperspectral reflectance observations will be taken at three day intervals following emergence on plants in the Root rot symptom experiment (above).