**CONTROL OF SOYBEAN DISEASES**

**TECHNICAL REPORT**

**NORTH DAKOTA SOYBEAN COUNCIL**

**JUNE 2018**

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Diseases are a potential problem in soybean production in North Dakota. The most important diseases that cause yield loss are those that affect the roots of soybean. A primary focus of this project is to work with the soybean breeder to incorporate host resistance to major diseases into public soybean varieties and germplasm. The two soil-borne diseases where sources of resistance are available are Phytophthora root rot and soybean cyst nematode (SCN). Also, we test a selection of commercial cultivars for resistance to SCN to provide growers additional information on the level of resistance. A second aspect of the research was to investigate changes in pathogen populations that would affect soybean production.

In cooperation with Dr. Helms, we continued to incorporate resistance into public soybean breeding lines. We maintain a variety of races of *P. sojae* in storage and each year the races we use for screening are grown in the laboratory, inoculated onto a set of plants with known resistance and susceptibility, and then re-isolated from infected plants to make sure they have maintained their known virulence. In addition we maintain other races which we use to determine which resistance genes are involved in the resistant reaction of plants. This past year most of the stored races were grown out in the laboratory and some were tested in the greenhouse for virulence. Cultures that are kept too long in storage can lose virulence. Some of the races had lost their virulence and were discarded. Only when we have verified the virulence of a particular race, is it used in the screening process. At present races 3 and 4 are the most common but other races exist in soybean fields. During 2018 we screened 60 breeding lines for resistance to race 4 and 56% of those lines were resistant. Another 21 lines were screened for resistance to race 3 and 57% were resistant. All screening is conducted in our greenhouse laboratory by injecting the pathogen into the stem of 9 day old plants and then placing the plants in a humid chamber for 48 hours. Susceptible plants usually wilt within 6 days. Many of the public varieties released by Dr. Helms have resistance to various races of *P. sojae*. Drs. Helms and Nelson have been cooperating on this research for over 30 years. In 2018 two conventional soybean varieties, Stutsman and ND Benson and two glyphosate resistant varieties, ND17009GT and ND18008GT, were released with resistance to Phytophthora root rot.

In addition to Phytophthora root rot we also screen NDSU breeding lines for resistance to SCN. In 2017-2018 we screened 14 advanced breeding lines for SCN resistance and 9 of those lines had high levels of resistance. One resistant line was released in 2018 as the conventional soybean variety ND Benson. We also screened 36 commercial round-up ready and 10 conventional soybean varieties for resistance to soybean cyst nematode HG 0. These tests were conducted under controlled conditions in the greenhouse. These were varieties that Dr. Helms had in his field testing program in 2016. We had tested these previously, but the tests were considered invalid due to some contamination of our HG 0 egg source with another HG type. Therefore we repeated the testing with a new source of HG 0 eggs. The results of those tests are reported in https://www.ag.ndsu.edu/varietytrials/fargo-main-station/2017-trial-results/2017-soybean-cyst-nematode-resistance-female-index-for-roundup-ready-and-conventional-varieties/view

In cooperation with Sam Markell, the extension pathologist we are investigating the possible occurrence of sudden death syndrome (SDS) in North Dakota soybean fields. SDS is a serious soil borne disease caused by *Fusarium virguliforme* and has not previously been identified in North Dakota. Dr. Markell’s project surveyed soybean fields for stem diseases and observed fields with symptoms similar to SDS. Stem samples were collected and given to our program to identify the pathogen. We also visited three of the fields with the most prominent symptoms to collect more root samples for processing. In addition we surveyed another 10 soybean fields in four counties where I had previously observed symptoms similar to SDS and in some of these fields SCN was also present which is often associated with SDS. In total, we collected plant samples from 15 fields where possible SDS foliar symptoms were observed.

To determine the presence of *F. virguliforme* in plant root samples showing SDS symptoms a qPCR assay was performed using the specific primers described by Wang et al. in 2014. Briefly, DNA was extracted from field samples using Mag-Bind Plant DNA Plus kit (Omega Bio-tek, Norcross, GA) and DNA concentration was determined using NanoDrop (Thermo Fisher, Waltham, MA). Three isolates of *F. virguliforme* (LL 0039 IA, FV wal-ss1, FV-Bel-SS4) were used as positive control in qPCR analysis. One primer set (F6-3 GTAAGTGAGATTTAGTCTAGGGTAGGTGAC and R6 GGGACCACCTACCCTACACCTACT) amplifying a 76-bp product was used with

SsoAdvanced™ Universal SYBR® Green Supermix (BioRad, Hercules, CA) and 2µl of fungal DNA under the following qPCR conditions: 95°C for 10 min (1 cycle), and 40 cycles at 95°C for 15 s and 60°C for 1 min, with fluorescent data collection in the annealing and extension step in the qPCR machine (BioRad, Hercules, CA) along with melt curve analysis. The qPCR assay detected the DNA of the positive *F. virguliforme* controls with Ct values ranging from 10.3 - 10.38. High Ct values in qPCR ranging between 25.55 to 31.3 were obtained for all the 2017 field samples except three which did not show any specific signal in qPCR. PCR products were then sent for sequencing to GenScript (Piscataway, NJ), but sequences did not provide a positive identification for *F. virguliforme*. Therefore, these results indicate that either plant samples were negative for *F. virguliforme* or fungal DNA was present in very low level. The symptoms observed in these fields may have been caused by other diseases such as brown stem rot or root rot caused by *F. solani*. In the summer of 2018 we will conduct a more intensive survey for sudden death syndrome and collect samples for pathogen verification.

In the summer of 2017 field experiments was established at the NDSU experiment station to evaluate the role of *F. solani* and *F. tricinctum* in “early senescence” or death of soybean in the later reproductive stages which is believed to be associated with root rot. Barnes soybean was grown in pots with three levels of inoculum (0, low and a moderate level) established in the soil. The pots were buried in the field with the bottoms removed and plants were grown under normal field conditions. Reductions in plant growth (plant height and plant dry weight) were observed during the seedling stage, especially at high inoculum levels (Table 1). There were plants that died from Fusarium but most died within 4 to 6 weeks after planting. The stunting of plant growth when infected with these Fusarium species was obvious in the seedling stage when inoculum levels were high. On the surviving plants four different measurements of plant growth along with two measurements of disease were recorded at or following harvest in October. The data are shown in Table 2. Although there was a solid trend in the data for a decrease in plant height, plant weight, and pod and seed weight by the two *Fusarium* species, the high variability in the data resulted in non- significant differences between the check and most of the Fusarium treatments. There was a significant decrease in plant height and seed weight caused by *F. tricinctum* at the high inoculum level. Lesion development on these plants indicated the pathogens had caused disease on the tap roots. The drier conditions early in the growth of the plants were favorable to disease development. Part of these field studies with *Fusarium* are partially supported by research funded by the United Soybean Board through a multistate cooperative research grant managed by Southern Illinois University.



Figure 1. Evaluating advanced breeding line ND10-3464 for resistance to race 4 of *Phytophthora sojae*. None of the ND10-3464 plants died while the results with the checks were as expected. ND10-3464 was released as the NDSU soybean variety ND Benson.

