Report to the Maryland Soybean Board – February 15, 2019

Identification of new sources of resistance/tolerance to *Sclerotinia sclerotiorum* among soybean germplasm showing resistance to *Phytophthora sojae*

Behnam Khatabi and Kate Everts

The first year of our project was completed successfully as outlined in the proposal. Our objectives were 1) characterize *S. sclerotiorum* isolates from soybean and other crops in the *Delmarva region,* 2) evaluate soybean germplasm for resistance to Sclerotinia stem rot under greenhouse condition, and 3) expand regional outreach activity to improve soybean stem rot management. Progress on each objective is outlined below.

Objective 1: Characterize *S. sclerotiorum* isolates from soybean and other crops in the *Delmarva region*

Forty *S. sclerotiorum* isolates were tested in 2018 for mycelial compatibility. Mycelial compatibility group (MCG) testing is a method to determine relatedness of fungal isolates, and how genetically homogenous the pathogen population is within a region. The MCGs were determined by pairing the isolates in all possible combinations on Diana Simmons (DS) medium (Cubeta et al., 2001) as described in Mandal and Dubey, 2012 and Zancan et al, 2015. A total of 750 combinations were obtained from 40 isolates and each pair was replicated twice. In addition, each isolate was paired with itself and a control (i.e. pure PDA plug). Before then MCG test, the isolates were grown on regular PDA and incubated at 24 ± 1 ^{O}C for one week. Mycelial discs (5 mm diameter) were taken from approximately 1 mm behind the advancing edge of actively growing mycelial colony on PDA and placed upside down on a plate of DS medium in Petri dishes (90 mm diameter) at 2.5 cm apart. Mycelial reactions were recorded after 7 days as incompatible when an apparent line of demarcation, a barrage zone, or a mycelia free zone is observed between the confronting paired isolates, and as compatible if there is no line of demarcation observed between the isolates. Radial growth of each isolate was also recorded to determine the growth or expansion performance of each isolate in the presence of the other isolate. The experimental design was completely randomized design (CRD) with 2 replications.

The MCG tests indicate that isolates in different regions comprise a heterogeneous mix of MCGs. No isolate was found to be compatible or incompatible with all other isolates. Compatible isolates were from different locations and hosts. MCGs on cultivated hosts are reported to be more complex, indicating that agricultural practices influenced MCG frequencies and patterns. In our experiment, out of 42 isolates collected from different locations/states and crops in the US, 12 major MCGs were identified.

Preliminary conclusion: The information that our population is similar, at least for MCG, to other populations is important for guiding management decisions. It is an indication that our populations of *S. sclerotiorum* in the Mid-Atlantic region are genetically similar to populations in other areas of the country.

Objective 2: To evaluate soybean germplasm for resistance to Sclerotinia stem rot under greenhouse condition.

We obtained approx. 200 seeds of each of the requested germplasm lines from Dr. Saghai Maroof (Virginia Tech), including several *Phytophthora sojae* resistant lines and several other elite cultivars, which were used as controlled comparison lines (for a total of 52 PI accessions and named lines). Seeds were planted in the field at the University of Maryland's Lower Eastern Shore Research and Education Center (LESREC) on June 14 in 5 ft. plots with 30 seed per plot in three replicates. The field had a moderate population of *Rhizoctonia solani*. Although evaluation of these lines for resistance to *R. solani* was not in the proposal, this was an opportunity to obtain additional information on these lines for this soil-borne disease. We also collected plant heights, and made observations on leaf and stem disease presence.

Field Evaluation:

Plants were visually assessed in the field for above-ground disease symptoms and plant vigor. A rating scale was used to assess individual plants. Plants were ranked on a disease severity index on a 1-5 scale, where 5 was the best (least disease symptoms) score. Disease severity and disease incidence were assessed by calculating the number of plants affected and the number of healthy-looking plants. The scale was: 5= Healthy plants throughout the plot, 4= Slight chlorosis, leaf spots and no impact on plant growth and few plants exhibiting symptoms, 3= Moderate chlorosis and disease symptoms several plants affected in the plot, 2= severe root rot disease symptoms and most or all plants affected. If a plot received a rating of 2, plants were selected and were subjected to the pathogen isolation (see below).

Table 1. Symptoms of soybean lines assessed in the field. Soybean lines are sorted from fewest to most symptoms present on the first rating date.

GERMPLASM LINE	FIRST FIELD SCORE[z]		SECOND FIELD SCORE		HEIGHT (CM)	
JACK	4.2[y]	A	3.3	ABCDE	35.8	ABC
PL88788	4.2	A	2.3	EFGH	30.2	DEFGHIJKL
36T36	4.0	AB	3.7	ABC	33.5	ABCDEF
L29	4.0	AB	3.0	BCDEF	32.0	ABCDEFGHI
PL408132	4.0	AB	3.7	ABC	33.0	ABCDEF
48T27	3.8	ABC	4.3	A	33.0	ABCDEF
LEE68	3.8	ABC	4.3	A	35.3	ABCD
PL408015	3.8	ABC	3.3	ABCDE	32.5	ABCDEFGH
39T28	3.7	ABCD	3.0	BCDEF	31.8	ABCDEFGHIJ
45T48	3.7	ABCD	3.7	ABC	30.8	BCDEFGHIJK
48A60	3.7	ABCD	4.0	AB	32.8	ABCDEFG
PL96983	3.7	ABCD	3.7	ABC	34.7	ABCDE
V94-5152	3.7	ABCD	4.0	AB	36.0	AB
11_PL398	3.5	ABCDE	2.0	FGHI	28.7	FGHIJKLMN
12_PL398	3.5	ABCDE	2.5	DEFG	25.5	KLMNOPQ
44T63	3.5	ABCDE	3.3	ABCDE	27.5	GHIJKLMNO

46T59	3.5	ABCDE	3.7	ABC	33.3	ABCDEF
PL361103	3.5	ABCDE	3.3	ABCDE	29.8	EFGHIJKL
PL399073	3.5	ABCDE	2.0	FGHI	27.5	GHIJKLMNO
PL399079	3.5	ABCDE	3.7	ABC	34.8	ABCDE
94Y23	3.3	BCDEF	3.3	ABCDE	30.5	CDEFGHIJKL
P408020A	3.3	BCDEF	2.3	EFGH	29.5	EFGHIJKLM
PL341264	3.3	BCDEF	3.7	ABC	36.3	A
PL407985	3.3	BCDEF	2.0	FGHI	25.3	LMNOPQR
WILLIAMS	3.3	BCDEF	2.3	EFGH	32.5	ABCDEFGH
ESSEX	3.2	CDEFG	2.7	CDEFG	27.3	HIJKLMNO
P408319C	3.2	CDEFG	3.7	ABC	31.5	ABCDEFGHIJ
PL319531	3.2	CDEFG	3.3	ABCDE	34.7	ABCDE
PL408029	3.2	CDEFG	3.0	BCDEF	28.8	FGHIJKLMN
PL408111	3.2	CDEFG	3.0	BCDEF	30.0	DEFGHIJKL
PL424477	3.2	CDEFG	4.0	AB	33.3	ABCDEF
P424237A	3.0	DEFGH	3.3	ABCDE	31.8	ABCDEFGHIJ
P424237B	3.0	DEFGH	3.3	ABCDE	30.0	DEFGHIJKL
PL157432	3.0	DEFGH	3.7	ABC	32.7	ABCDEFGH
PL200543	3.0	DEFGH	3.7	ABC	29.7	EFGHIJKLM
PL398666	3.0	DEFGH	2.3	EFGH	24.3	MNOPQRS
PL398996	3.0	DEFGH	1.0	I	15.7	TU
PL408097	3.0	DEFGH	3.0	BCDEF	30.0	DEFGHIJKL
PL408287	3.0	DEFGH	1.7	GHI	23.7	NOPQRS
PL200553	2.8	EFGH	4.3	A	33.7	ABCDEF
P567139B	2.7	FGH	4.3	A	34.8	ABCDE
PL423741	2.7	FGH	2.0	FGHI	13.7	U
CNS	2.5	GH	3.7	ABC	25.8	KLMNOP
PARKER	2.5	FGH	Missing data		20.0	RST
PL398440	2.5	GH	2.0	FGHI	20.2	QRST
PL399004	2.5	GH	2.7	CDEFG	26.7	IJKLMNOP
YORK	2.5	FGH	3.5	ABCD	28.8	EFGHIJKLMNO
PL274508	2.3	H	2.3	EFGH	26.5	JKLMNOP
PL398775	2.3	H	1.3	HI	19.5	ST
PL398791	2.3	H	1.3	HI	21.8	PQRS
PL398946	2.3	H	2.3	EFGH	23.2	OPQRS
***P* value**[x]	0.0001		0.0001		0.0001	

[z]Rating scale 5= Healthy plants throughout the plot, 4= Slight chlorosis, leaf spots and no impact on plant growth and few plants exhibiting symptoms, 3= Moderate chlorosis and disease symptoms several plants affected in the plot, 2= severe root rot disease symptoms and most or all plants affected.

[y]Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).

[x]*P* value ≤ 0.05 indicates significant differences among treatments.

Identification of Root Associated Fungi:

In addition to evaluation of disease susceptibility in the field, we isolated and are identifying the fungi associated with the diseases soybean roots. These fungi may play a role in reducing plant health. About 5-10 pieces of soybean roots showing root rot disease were collected from the LESREC soybean field by the Khatabi lab. Soybean roots were washed, air dried, surface sterilized in 1% sodium hypochlorite solution for 3 min, washed several times with sterilized distilled water and then dried between two sterilized filter papers. The root fragments were aseptically transferred to the surface of plates of Potato dextrose agar medium (PDA a general-purpose medium for isolation and cultivation of fungi). Plates were incubated at 25°C for 5 days and were examined daily. The developed mycelial growth was picked up and transferred onto new PDA medium. Purification of each isolated fungus was carried out by excising the mycelial tip and transferring it to a clean PDA plate. Stock cultures were maintained on PDA slants and kept at 5°C.

A total 17 fungal isolates were obtained and subjected to identification using 18S rRNA gene, a universal eukaryote specific primer. Fungal isolates were grown on PDA medium and incubated for 24 h at 28 °C and used as starting material for total DNA extraction. DNA extraction was carried out by using genomic fungal DNA purification kit. The gene encoding internal transcribed spacer (ITS) of nuclear ribosomal DNA (ITS1 or ITS2) was amplified by PCR with the 18S fungal specific primers 27F (5'- CAG CCG CGG TAA TTC C -3') and 1200R (CCC GTG TTG AGT CAA ATT AAG C-3'), which resulted in the amplification of an approximately 650 bp PCR product (Hadziavdic et al., 2014). Amplification was performed using PCR in 25 µL reaction mixture containing 0.4 µL DNA (50 µg ml^{-1}), 2.5 µL 10X PCR buffer, 0.5 µL dNTPs (2 mM), 0.5 µL of each primer (10 pmol), 0.2 µL Taq DNA polymerase (5 U μL^{-1}). After a denaturation step of 5 min at 94°C, the amplification reactions were performed, with 30 cycles of denaturation (60 s, 94°C), primer annealing (60 s, 55°C), and primer extension (60 s, 72°C) and a final extension step of 7 min at 72°C. PCR amplicons were analyzed by electrophoresis in 1.2 % (w/v) agarose gel with a molecular size marker 100 bp, followed by staining with ethidium bromide. Electrophoresis was carried out in in the 1× TBE buffer (0.09 M Tris base, 0.09 M sodium borate, 2.5 mM EDTA, pH 8.3) at 100 V for 1 h. Then, the gel was visualized under UV light and documented using gel Imager).

Sequencing and Phylogenetic Analysis of unknown fungi will be conducted as follows. The 18S rDNA sequences of all isolates will be aligned with reference sequences showing sequence homology from the NCBI database using the multiple sequence alignment program of MEGA 4.0. The evolutionary distances will be computed using the software package TREECON version 1.3. The construction of neighbor-joining tree and program analysis of 1000 re-samplings will be carried out using program MEGA 4.0. Identification will be based on the results obtained from sequencing and phylogeny the phylogenetic analysis.

***Sclerotinia sclerotiorum* susceptibility and aggressiveness test greenhouse:**

An experiment was also conducted in the Plant Science and Landscape Architecture greenhouse at University of Maryland College Park. Germplasm lines were grown in the research greenhouse, in controlled conditions, inoculated with *S. sclerotiorum* and rated for the disease severity. In addition to the lines we received from Dr. Maroof, an extra 30 soybean accessions were requested from the

USDA Soybean Germplasm Collection through the Germplasm Resources Information Network (GRIN) website and were used to score the lines for susceptibility to Sclerotinia stem rot.

Seed of seventy-nine soybean lines were planted during fall of 2018 in the greenhouse for the screening test (Table 2). The seeds were planted into a substrate containing perlite and peat mix in sterile pots of 15 cm size and placed on a greenhouse bench. After germination, plants were fertilized with a solution of 15-5-15 100ppm fertilizer and water at 250 ppm three times per week until the plants were developed. The greenhouse temperatures were maintained at 20 ± 1°C (night, 12 h) and 26 ± 1°C (day, 12 h). Supplemental greenhouse daylight of 12 hours each day was maintained. We planted the different lines at 4-day intervals and inoculated with 2 isolates of *S. sclerotiorum* after one month, or when plants start developing the 5th node. The days were considered blocks and the experimental design was a randomized complete block design with three replicates of each line for the 2 isolates. Aggressiveness or straw test was conducted as described by Otto-Hanson et al. (2011) and modified by Zancan et al, 2015.

Table 2. Planting date, disease inoculation date and measurement date of greenhouse evaluation.

Lines	Replication	DAY OF PLANTING	DAY OF INOC.	DAY OF DISEASE MEASU.
from 1 - 52	1	9/14/2018	10/14/2018	10/22/2018
	2	9/17/2018	10/17/2018	10/25/2018
	3	9/18/2018	10/18/2018	10/26/2018
	4 (control)	9/25/2018	10/25/2018	11/2/2018
53 - 79	All (1 - 4) reps	10/24/2018	11/24/2018	12/2/2018

For inoculation, sterile drinking straws of approximately 5 mm in diameter and 2 cm long were used. One end of the straw was heat sealed and the other end was used to bore into the leading edge of a growing culture of *S. sclerotiorum*. The open end of the straw was infiltrated into the reverse side of seven days old *S. sclerotiorum* culture on PDA at the advancing edge of the mycelia of each isolate. The stem of each plant was cut 2 cm above the fourth node (i.e. the internode between the fourth and fifth node) and the straw containing agar and fungal mycelium was placed over the cut stem. During and after inoculations, we maintained 20°C nighttime and 26°C daytime temperatures in greenhouse. The inoculated plants were incubated for 8 days and during the first 48 hours they were misted to keep the leaves wet. The development of lesions was evaluated by measuring the lesion length/size using a ruler (Figure 1). The mean of 3 plants was used for the analysis of variance.

Two isolates were used to inoculate the plants because variability exists in aggressiveness of *S. sclerotiorum* isolates. We selected SS27, originally isolated from Lima bean, and SS29, originally isolated from a tomato.

Figure 1. Graduate student Habtamu Demmisie measuring the lesion length in the greenhouse.

The soybean germplasm lines varied in susceptibility to the two *S. sclerotiorum* isolates, as expected. Some lines were very susceptible to one isolate and not another (the same data is shown in graphically in Figure 2 and numerically in Table 3). However, several lines had relatively small lesions to both isolate SS27 and SS29. PI 398249, PI 96983, PI 398666, and V945152 had shorter lesion lengths than Williams, the susceptible control to both isolates (Figure 2 and Table 3, see bold entries).

Figure 2. Length of lesions on soybean germplasm lines inoculated with *S. sclerotiorum* isolates SS27 or SS29 and incubated in the greenhouse for 8 days.

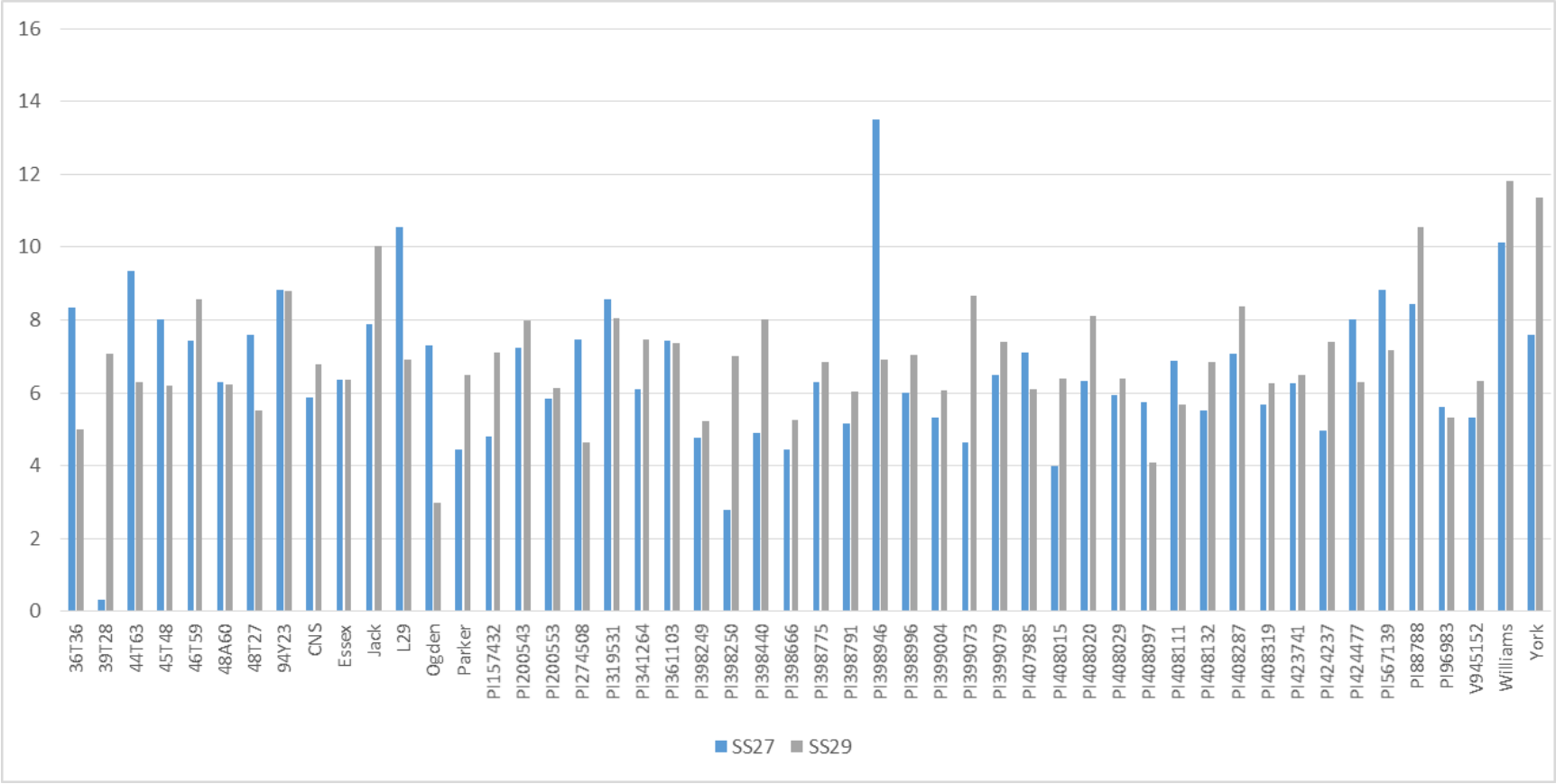

Table 3. Lesion size of soybean plants inoculated in the greenhouse to two isolates of *Sclerotinia sclerotiorum* in the fall of 2018.

GERMPLASM LINE	ISOLATE	ESTIMATE	LETTER GROUP
36T36	SS27	8.3[y]	CDEFGHIJK
36T36	SS29	5.0	JKLMNOPQRS
39T28	SS27	0.3	T
39T28	SS29	7.1	EFGHIJKLMNOPQ
44T63	SS27	9.3	BCDEF
44T63	SS29	6.3	FGHIJKLMNOPQR
45T48	SS27	8.0	CDEFGHIJKLM
45T48	SS29	6.2	FGHIJKLMNOPQRS
46T59	SS27	7.4	DEFGHIJKLMNOPQ
46T59	SS29	8.6	BCDEFGHI
48A60	SS27	6.3	FGHIJKLMNOPQR
48A60	SS29	6.2	FGHIJKLMNOPQRS
48T27	SS27	7.6	CDEFGHIJKLMNOPQ
48T27	SS29	5.5	GHIJKLMNOPQRS
94Y23	SS27	8.8	BCDEFG
94Y23	SS29	8.8	BCDEFG
CNS	SS27	5.9	GHIJKLMNOPQRS
CNS	SS29	6.8	EFGHIJKLMNOPQ
ESSEX	SS27	6.4	FGHIJKLMNOPQR
ESSEX	SS29	6.4	FGHIJKLMNOPQR
JACK	SS27	7.9	DEFGHIJKLMN
JACK	SS29	10.0	ABCDE
L29	SS27	10.5	ABCD
L29	SS29	6.9	EFGHIJKLMNOPQ
OGDEN	SS27	7.3	DEFGHIJKLMNOPQ
OGDEN	SS29	3.0	RST
PARKER	SS27	4.4	LMNOPQRS
PARKER	SS29	6.5	FGHIJKLMNOPQ
PI157432	SS27	4.8	LMNOPQRS
PI157432	SS29	7.1	DEFGHIJKLMNOPQ
PI200543	SS27	7.2	DEFGHIJKLMNOPQ
PI200543	SS29	8.0	CDEFGHIJKLM
PI200553	SS27	5.8	GHIJKLMNOPQRS
PI200553	SS29	6.1	FGHIJKLMNOPQRS
PI274508	SS27	7.5	DEFGHIJKLMNOP
PI274508	SS29	4.6	MNOPQRS
PI319531	SS27	8.6	BCDEFGHI
PI319531	SS29	8.0	CDEFGHIJKLM
PI341264	SS27	6.1	FGHIJKLMNOPQRS
PI341264	SS29	7.5	DEFGHIJKLMNOP

PI361103	SS27	7.4	DEFGHIJKLMNOPQ
PI361103	SS29	7.4	DEFGHIJKLMNOPQ
PI398249	**SS27**	**4.8**	**LMNOPQRS**
PI398249	**SS29**	**5.2**	**HIJKLMNOPQRS**
PI398250	SS27	2.8	ST
PI398250	SS29	7.0	EFGHIJKLMNOPQ
PI398440	SS27	4.9	KLMNOPQRS
PI398440	SS29	8.0	CDEFGHIJKLM
PI398666	**SS27**	**4.4**	**OPQRS**
PI398666	**SS29**	**5.3**	**HIJKLMNOPQRS**
PI398775	SS27	6.3	FGHIJKLMNOPQR
PI398775	SS29	6.8	DEFGHIJKLMNOPQR
PI398791	SS27	5.2	IJKLMNOPQRS
PI398791	SS29	6.0	FGHIJKLMNOPQRS
PI398946	SS27	13.5	A
PI398946	SS29	6.9	EFGHIJKLMNOPQ
PI398996	SS27	6.0	FGHIJKLMNOPQRS
PI398996	SS29	7.1	DEFGHIJKLMNOPQ
PI399004	SS27	5.3	JKLMNOPQRS
PI399004	SS29	6.1	FGHIJKLMNOPQRS
PI399073	SS27	4.6	MNOPQRS
PI399073	SS29	8.7	BCDEFGH
PI399079	SS27	6.5	FGHIJKLMNOPQ
PI399079	SS29	7.4	DEFGHIJKLMNOPQ
PI407985	SS27	7.1	DEFGHIJKLMNOPQ
PI407985	SS29	6.1	FGHIJKLMNOPQRS
PI408015	SS27	4.0	QRS
PI408015	SS29	6.4	FGHIJKLMNOPQR
PI408020	SS27	6.3	FGHIJKLMNOPQR
PI408020	SS29	8.1	CDEFGHIJKL
PI408029	SS27	5.9	FGHIJKLMNOPQRS
PI408029	SS29	6.4	FGHIJKLMNOPQR
PI408097	SS27	5.7	GHIJKLMNOPQRS
PI408097	SS29	4.1	PQRS
PI408111	SS27	6.9	DEFGHIJKLMNOPQ
PI408111	SS29	5.7	GHIJKLMNOPQRS
PI408132	SS27	5.5	GHIJKLMNOPQRS
PI408132	SS29	6.8	EFGHIJKLMNOPQ
PI408287	SS27	7.1	EFGHIJKLMNOPQ
PI408287	SS29	8.4	BCDEFGHIJ
PI408319	SS27	5.7	GHIJKLMNOPQRS
PI408319	SS29	6.3	FGHIJKLMNOPQR
PI423741	SS27	6.3	FGHIJKLMNOPQR
PI423741	SS29	6.5	FGHIJKLMNOPQ
PI424237	SS27	5.0	NOPQRS

PI424237	SS29	7.4	EFGHIJKLMNO
PI424477	SS27	8.0	CDEFGHIJKLM
PI424477	SS29	6.3	FGHIJKLMNOPQR
PI567139	SS27	8.8	BCDEFG
PI567139	SS29	7.2	DEFGHIJKLMNOPQ
PI88788	SS27	8.4	BCDEFGHIJ
PI88788	SS29	10.5	ABCD
PI96983	**SS27**	**5.6**	**GHIJKLMNOPQRS**
PI96983	**SS29**	**5.3**	**HIJKLMNOPQRS**
V945152	**SS27**	**5.3**	**HIJKLMNOPQRS**
V945152	**SS29**	**6.3**	**FGHIJKLMNOPQR**
WILLIAMS	SS27	10.1	ABCDE
WILLIAMS	SS29	11.8	AB
YORK	SS27	7.6	DEFGHIJKLMNO
YORK	SS29	11.4	ABC
P VALUE[X]		0.0001	

[y]Means within a column followed by the same letter are not significantly different according to Fisher's protected LSD test ($\alpha = 0.05$).
[x]*P* value $\leq$ 0.05 indicates significant differences among treatments.

Table 4. Sclerotinia stem rot lesion length in greenhouse grown soybean germplasm lines planted on Oct 24, 2018 (lines 53 to 79, all replicates). P value of 0.0897 indicates that no significant differences were observed.

Line number	Lesion length (cm)
PI438477	5.25
PI536636	5.25
PI438471	5.43
PI642055	5.50
PI297543	6.60
PI548317	6.78
PI548667	6.87
PI573008	6.90
PI547402	7.03
PI548379	7.17
PI548595	7.17
PI483084	7.32
PI556897	7.58
PI506764	7.86
PI548609	7.90
PI559369	8.05
PI548663	8.22
PI548631	8.33
PIPI5530	8.38

PI424131	8.70
PI548533	8.98
PI548477	9.53
PI561398	9.83
PI596752	9.83
PI553039	11.28
PI438497	11.63
PI561394	19.48
P= 0.0897	

Objective 3: Expand regional outreach activity to improve soybean S. sclerotiorum management.

Our final objective proposed to conduct outreach to Maryland growers on *S. sclerotiorum*. Several talks were given on *Sclerotinia sclerotiorum* by Habtamu Demissie.

1. Crops Twilight Tour CMREC Upper Marlboro Farm, Aug. 8, 2018
2. Farmers' Field Day at LESREC Salisbury, Wednesday, June 27, 2018
3. Southern Maryland Fruit and Vegetable Meeting, Leonardtown, Feb. 7, 2019
4. Delaware Agriculture Week, Processing Vegetable Session, Harrington, Jan. 16, 2019.

In addition, the University of Delaware recently hired Dr. Alyssa Koehler to conduct research and extension in Delaware and Maryland on field crops. We have begun a close collaboration with Dr. Koehler to expand our research on Stem rot on soybean. (Dr. Koehler shares an interest in *S. sclerotiorum* and has conducted research on this pathogen on Stevia). We are enthusiastic about Dr. Koehler's arrival and will work closely with her to leverage our mutual interest in Sclerotinia, to benefit soybean growers, and to position our research for improved national funding.

References:

Cubeta, M. A., Sermons, D. N., Cody, B. R. (2001). Mycelial interactions of *Sclerotinia minor*. (Abstr.). Phytopathology, 91: S19.

Mandal, A. K., and Dubey, S. C. (2012). Genetic diversity analysis of *Sclerotinia sclerotiorum* causing stem rot in chickpea using RAPD, ITS-RFLP, ITS sequencing and mycelial compatibility grouping. World J. Microbiol Biotechnol, 28: 1849 – 1855.

Hadziavdic, K., Lekang, K., Lanzen, A., Jonassen, I., Thompson, E. M., & Troedsson, C. (2014). Characterization of the 18S rRNA gene for designing universal eukaryote specific primers. *PloS one*, *9*, e87624.

Otto-Hanson, L., Steadman, J. R., Higgins, R., and Eskridge, K. M. 2011. Variation in Sclerotinia sclerotiorum bean isolates from multisite resistance screening locations. Plant Dis. 95:1370-1377.

Zancan, W. L. A., Steadman, J. R., Higgins, R., Jhala, R., Machado, J. da C. (2015). Genetic and aggressiveness variation among *Sclerotinia sclerotiorum* dry bean isolates from Brazil fields. Biosci. J., Uberlândia, 31(4), 1143 – 1151.