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Developing Management Strategies for Taproot Decline, Xylaria sp., in Soybean

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DEVELOPING MANAGEMENT STRATEGIES FOR TAPROOT DECLINE, *XYLARIA* SP., IN SOYBEAN

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of
Plant Pathology and Crop Physiology

by
Myra A. Purvis
B.S. Louisiana Tech University, 2003
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ABSTRACT

Soybean (*Glycine max*) is one of the most important oilseed crops in the world. Taproot decline is a recently discovered disease caused by *Xylaria sp.*, a novel species located within the *Xylaria arbuscula* aggregate. Foliar symptoms include interveinal chlorosis and necrosis, and upon further investigation, there are often dead plants adjacent within the row. Many other soybean diseases have similar foliar symptoms; therefore, more examination is usually required for proper identification. Soybean debris from previous years is suspected to be the primary source of inoculum. Plants may be infected at any point during the growing season, often resulting in premature death. Precision planting, reduced tillage, and soybean monoculture may contribute to disease incidence and severity. There is little knowledge of genetic resistance, fungicide efficacy, or cultural practices that may be useful in managing taproot decline.

In greenhouse trials we have identified susceptible, moderately susceptible, moderately resistant, and resistant soybean varieties for growers. Limited field data appears to corroborate these results, and more research is needed. To date, no promising seed treatments have been identified in the field. However, a few promising in-furrow fungicide treatments have been identified in field trials. Results from on-farm studies indicate that taproot decline causes significant yield loss. Results from these projects will directly benefit Louisiana stakeholders by providing potential management options for taproot decline.

CHAPTER 1. INTRODUCTION

Importance of Soybean Soybean is one of the most important oilseed crops grown in over 70 countries (Hartman et al. 2015). The United States (US) was the leading soybean producer in 2017, with Louisiana ranking 18th, producing 62.4 million bushels. This is a value of over $654 million to the Louisiana economy (Quick Stats, USDA-NASS, 2017).

Importance of Taproot Decline Taproot decline (TRD) is a soybean disease caused by a previously undescribed species within the genus, *Xylaria* (Allen et al. 2017). The disease caused yield losses of approximately 770,000 bushels in Louisiana in 2017, and is widespread in the southern US (Allen et al. 2018). The disease has been identified in Alabama, Arkansas, Louisiana, Mississippi, and Tennessee (Figure 1.1) (Allen et al. 2017). Some specialists believe TRD is found further north along the Mississippi River Valley, but that has yet to be officially verified (personal communication). Anecdotal evidence indicates higher incidence and severity in soybean monoculture and reduced tillage systems.

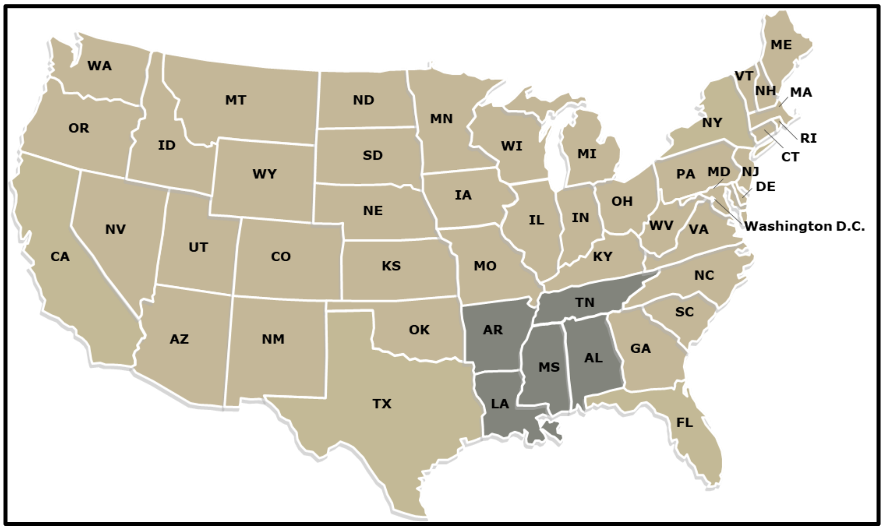

Figure 1.1. States where taproot decline has been reported (gray).

Importance of Xylaria *Xylaria* was first observed within soybean seed from Ethopia in 1979 (Mengistu and Sinclair) and was one of many organisms isolated in that study. The first report on soybean of our *Xylaria* sp. causing TRD was in March of 2017 by Allen et al. The pathogen is most closely related to *Xylaria striata,* within the *Xylaria arbuscula* aggregate (Allen et al. 2017). *Xylaria* spp. are generally considered saprophytes of wood and other plant parts (Allen et al. 2017); however, some species are known pathogens on apple and cherry trees (Rogers, 1984). Some *Xylaria* spp. are endophytes, such as those inhabiting *Pinus strobus* needles in Nova Scotia (Richardson et al. 2014). Other endophytes within this genus have been reported in peach pits and magnolia litter (Rogers et al. 2002).

Symptoms, Signs, and Epidemiology Symptoms of TRD are usually noticed during pod fill and include interveinal chlorosis and necrosis, which is usually indicative of a root or lower stem issue (Hartman et al. 2015). The color of foliar symptoms may vary from yellow to orange with location and variety (Figure 1.2, Allen et al. 2017). This foliar symptom may be mistaken

Figure 1.2. Soybean plants exhibiting symptoms of TRD.

for many other soybean diseases and maladies including sudden death syndrome (*Fusarium virguliforme*), root knot nematode (*Meloidogyne incognita*), red crown rot (*Cylindrocladium crotalariae*), stem canker (*Diaporthe* sp.), and fungicide toxicity (Hartman et al. 2015). With the increase of reduced tillage and soybean monoculture, seed are often planted into stubble from the previous season. Accurate planting systems and tractors with guidance place the seed in the same furrow year after year (Figure 1.3). This puts the soybean seed in direct contact with colonized plant tissue from previous seasons. In turn, infection can occur early in the growing season causing seedling and/or vegetative stage death. Dead plants are often overlooked, and symptoms observed later in the season manifest around these focal points. When infected plants are excised from the soil, roots are usually in contact with soybean debris colonized by *Xylaria* sp. from previous years. Plants expressing symptoms of TRD can be linearly clustered within the row (Allen et al. 2017). The characteristic symptom of TRD is a blackened taproot, where *Xylaria* sp. forms black stroma on the root surface (Figure 1.4). Another sign of TRD is white, cottony mycelial growth within the pith near the crown, which is best observed when the stem is split longitudinally. A sign of *Xylaria* sp. that is observed in affected fields during periods of

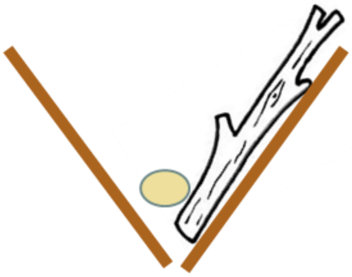

Figure 1.3. Soybean in a furrow contacting stubble from the previous year.

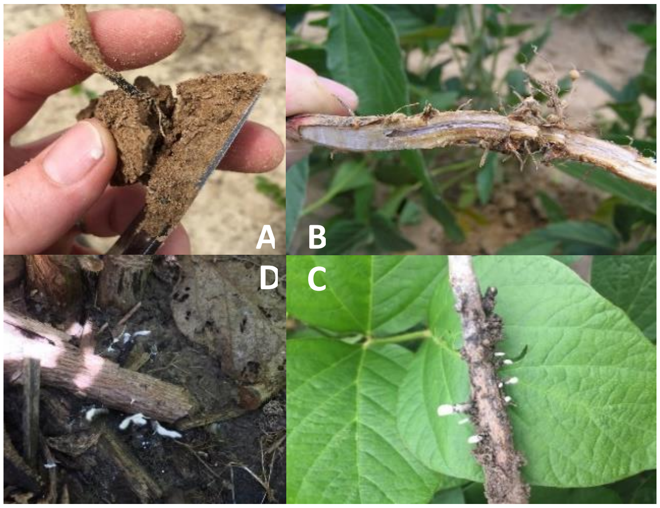

Figure 1.4. Characteristic symptoms of TRD: blackened taproot (A), white mycelia (B), and stromata (C and D).

high humidity are “dead man’s fingers”, or stromata, which are compact masses of mycelia that support fruiting bodies. For many years, this disease was misidentified as black root rot, caused by *Thielaviopsis basicola*, and also was referred to as the “mystery disease” (Allen et al, 2017).

Soybean producers need information concerning yield losses, varietal resistance, chemical control, and the effect of cultural practices on TRD. Therefore, the objectives of this research project were: to screen for varietal resistance in the greenhouse and in the field, to determine the efficacy of seed treatments and in-furrow fungicides, and to determine yield losses associated with TRD.

CHAPTER 2. RESPONSE OF SOYBEAN VARIETIES TO *XYLARIA* SP., CAUSAL AGENT OF TAPROOT DECLINE

Pathogen Isolation Infected soybean plants were removed from a field near Winnsboro, LA, where taproot decline (TRD) was observed. Isolation of *Xylaria* sp. was accomplished by removing the tops of the plants 7.5 cm above the crown, washing roots under running water for 15 minutes, surface-sterilizing tap and lateral root sections with 1:10 sodium hypochlorite solution for 1 minute then rinsing in tap water for 15 seconds. Under an EdgeGARD laminar flow hood (The Baker Company, Sanford, ME, USA), lateral roots were cut into 1 cm sections, placed into 1:10 solution of sodium hypochlorite for 45-75 seconds, and rinsed in sterile, distilled water for 1 minute. Lateral root sections were then placed on potato dextrose agar (Cole-Parmer, Inc. Vernon Hills, IL, USA) amended with chloramphenicol (75 ppm) and streptomycin sulfate

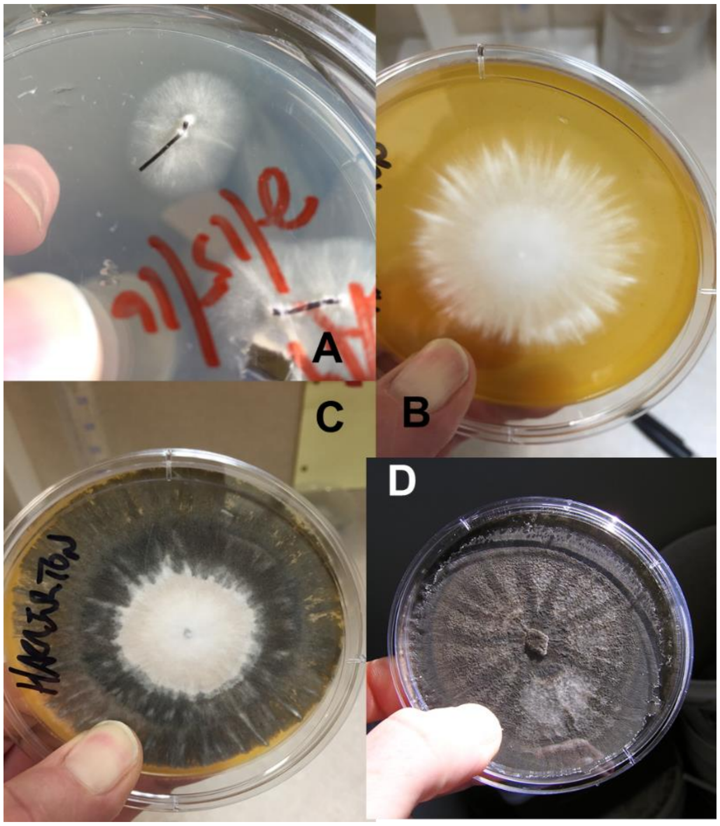

Figure 2.1. Colony characteristics. Surface sterilized roots plated (A), initial growth (B), 7 day old culture (C), and 14 day old culture (D).

(125 ppm). Isolation from taproots was accomplished by splitting surfaced sterilized roots and lower stems with a sterilized scalpel and directly transferring white mycelial growth to PDA-CS under a laminar flow hood. Plates were placed into an incubator set to deliver a 12 hour light:dark cycle at room temperature (21-24^{o}C) for 7 days. Colonies were initially white, but within about 14 days became grayish-black with black stroma on the underside. Colonies were circular in form with a starburst pattern (Allen et al. 2017) (Figure 2.1).

Inoculum Production The same *Xylaria* sp. isolate (MSU_SB201401) that was used to confirm pathogenicity (Allen et al. 2017) was transferred (1 cm plugs) from PDA-CS to Erlenmeyer flasks containing sterilized (121°C, 25 min) soybean flour (3.75 g), corn meal (3.75 g), sucrose (7.5 g), calcium carbonate (0.75 g), stir magnet, and 250 mL of distilled water (Walker and Boyette, 1985). Liquid cultures were allowed to grow on the lab bench for 7 days in ambient lighting on a magnetic stirrer at approximately 21-24^{o}C. Japanese millet, *Echinochloa esculenta,* (Wax Seed Co., Amory, MS, USA) (2.3 kg) was placed into a Unicorn Manufacturing (Plano, TX, USA) vented (0.2 µm) autoclave bag (25x13x61 cm) with 1.9 L of water then autoclaved at 121^{o}C for 60 minutes, allowed to cool 6-12 hours, then sterilized again. After cooling to less than 30°C, 50 ml of liquid *Xylaria* sp. culture was added to the millet. Millet-based cultures were allowed to colonize for 12-14 days at 21-24^{o}C and thoroughly mixed by shaking every 4-5 days. Infested millet was evenly spread (0.5 cm deep) on tables covered with Kraft paper (Uline, Pleasant Prairie, WI, USA) for 7-10 days under ambient lighting at 21-24°C, with hand stirring every 2-3 days. Inoculum was stored in a refrigerator (4^{o}C) in large paper grocery bags (Uline, Pleasant Prairie, WI, USA) until use. This method of inoculum production was used in all field and greenhouse studies.

Greenhouse Setup Experiments were conducted in a greenhouse facility near Winnsboro, LA, at the LSU AgCenter Macon Ridge Research Station (MRRS). Soybean seed were planted in Sunshine Professional Growing Mix #8 (Sun Gro Horticulture, Agawam, MA, USA) supplemented with Osmocote 14-14-14 slow release fertilizer (Everris NA, Inc., Dublin OH, USA) in 10 or 15 cm pots with a furrow pressed into the potting soil across the diameter of pots using a specialized steel tool. Seeds were sown evenly across the furrow, 4 (variety screening) or 6 (inoculum concentration) per pot, and either inoculated or not, according to the treatment design. Pots were placed on fiberglass, flood-irrigated tables and watered twice daily for 15 minutes at 8am and 8pm. Soybean plants were grown under supplemental lighting (Welthink LED, Hangzhou, China) with a 12-h light:dark cycle. Greenhouse temperatures ranged from 15.5 to 27^{o}C, and experiments were conducted during the fall/winter months.

Data Collection At 21 days after planting (DAP), emergence was counted, plants were removed from pots, roots washed of potting soil, and weighed. Roots and shoots were weighed separately by cutting the plants at the crown, weighing them, and placing them into paper bags to dry in a drying room for 7 days at 52^{o}C prior to determining final root and shoot weights.

Inoculum Concentration Before determining varietal reaction to *Xylaria* sp., inoculum concentration was standardized. Fifteen cm pots were filled with growth media, and furrows were created as previously described. The pots were inoculated with 0, 1, 2, 4, 6, 8, or 10 cc/15 cm furrow of inoculum prepared as previously described. Pots were placed on flood-irrigated fiberglass tables, and plants were grown under the previously described conditions. Plants were harvested at 21 DAP, and emergence, root weights, and shoot weights were obtained as previously described. The experiment was repeated three times in a randomized complete block design with 4 replications using a known susceptible soybean variety, AsGrow 4632. Data were subjected to ANOVA (JMP Pro 14, SAS, Cary, NC, USA). Means were compared to the non-treated control using Dunnett's *post hoc*.

***Varietal Response to Xylaria* sp.** Seed of soybean varieties (n=147) (Table 2.1) were obtained from the 2016 Official Variety Trial (OVT) supply where entries are submitted by multiple seed companies for evaluation by LSU AgCenter scientists. The varieties provided had seed treatments, however, no evidence has been provided that seed treatment affects TRD. Because of space and time constraints, 10 cm pots were used instead of 15 cm, and the inoculum amount was reduced to 0.67 cc/10 cm furrow. Instead of six, four seed were planted in the same growth medium as previously described. One experiment, or "run" consisted of 40 varieties, inoculated with corresponding non-inoculated controls and arranged in a randomized complete block design with four replicates. Twenty-seven varieties were screened on the last run. Each experiment was repeated once. Data were subjected to analysis of variance using JMP Pro 14. Run and replication were considered random effects. Variety and inoculation were considered fixed effects. Significant effects for variety ($P<0.0001$) and inoculation ($P<0.0001$) were observed. Based on non-significant run x variety interactions ($P=0.4382$), data were combined. Means for emergence, root, and shoot weight reduction were compared for individual varieties using a paired t-test ($\alpha=0.05$).

Table 2.1. List of seed companies and varieties, LSU AgCenter Official Variety Trials, 2016.

University of Arkansas	Progeny Ag Products	Stratton Seed	Bayer CropScience	Dyna-Gro Seed	Croplan	Mycogen Seed	Delta Grow Seed
OSAGE	P5555RY	GS47R216	CZ 4540LL	S49XT07	R2C4775	5N406R2	4880 RR
R10-197RY	P4788RY	Go Soy 5515LL	CZ 5515LL	S43RY95	RX4825	5N414R2	5625 RR2
UA 5612	P4900RY	Schillinger 5220.RC	CZ 5147LL	S48RS53	RX4926	5N490R2	5230 RR2
UA 5213C	P5289RY	Go Soy 4915R2	CZ 3841LL	S52LL66		5N452R2	4825 RR2
R10-230	P4211RY	Go Soy 5214GTS	CZ 4656RY	S52RY77		5N424R2	4670RR2
UA 5014C	P4814LLS	Go Soy 5115LL	CZ 5242LL	S57RY26		5N523R2	5067 LL
UA 5414RR	P4247LL	Schillinger 557.RC	CZ 4748LL	S56RY84		5N480R2	4967 LL
R07-6614RR	P5414LLS	GS45R216	CZ 4181RY	S48XT56		5N433R2	4970 RR
UA 5814HP	P5226RYS	Go Soy 49G16	CZ 3945LL	S42RY77			4995 RR
R09-430	P4757RY	GS48R216	CZ 4222LL	S49LL34			4790 RR2
	P4930LL	Go Soy 4714GTS	CZ 4818LL	S45XS66			5170 RR2
	P4613RY	Go Soy IREANE	CZ 4959RY	S47RY13			4587 LL
	P4588RY	Go Soy 4913LL	CZ 5445LL				5461 LL
	P5752RY	Go Soy LELAND	CZ 4898RY				5520RR2
			CZ 5150LL				DGX4845 RR2
			CZ 4590RY				
			CZ 4105LL				
			HBKLL4953				
			CZ 5375RY				
			CZ 4044LL				
			CZ 5225LL				
			CZ 3991RY				

(table cont’d)

Terral Seed	Asgrow-Monsanto	Armor Seed	University of Missouri	Syngenta	DuPont Pioneer
51A56	AG 44X6	47-R70	S12-2418	S39-T3	P41T33R
57R21	AG 55X7	49-D90	S12-3782	S47-K5	P47T36R
56R63	AG 45X6	49-D66	S12-3791	S39-C4	P54T94R
49R94	AG 46X6	ARX4906	S11-17025	S55Q3	P47T89R
49A75	AG 46X7	47-D17	S11-20124	S45W9	
47R34	AG 48X7	ARX4706	S12-4718	S42-P6	
52A95	AG 47X6	46-D08			
48A76	AG 49X6	48-D24			
48L63	AG 54X6	48-D80			
49L49	AG 53X6	55-R68			
47016R	AG59X7	ARX5506			
48A26		50-D04			
45A46					

Field Confirmation For the field screening project, the seed source is as previously described. In May of 2016, 32 varieties chosen at random were planted at MRRS in Winnsboro, LA. In May 2017 and 2018, 16 susceptible and 16 resistant varieties, based on preliminary greenhouse data, were planted at MRRS in Winnsboro. Agronomic milestones are identified in Table 2.2. All field trials were planted in a randomized complete block design with 4 replications. Plots consisted of 2 rows, 20 ft in length, with one row inoculated (2cc/row ft). Parameters measured include: emergence, taproot decline incidence (%), mortality (%), plant height (in), and yield (bu/a). Data were subjected to analysis of variance using JMP Pro 14 (SAS, Cary, NC, USA). Overall analysis indicated differences among varieties for all measured parameters. Therefore, means for individual varieties were compared using a paired t-test (α=0.05).

Table 2.2. Agronomic milestones for field confirmation locations during 2016-2018 at MRRS near Winnsboro, Louisiana.

Location	Planting Date	Soil Type	Rainfall (inches)	Harvest Date
Winnsboro, LA	April 26, 2016	Gigger-Gilbert silt loam	20.49	October 6, 2016
Winnsboro, LA	May 17, 2017	Gigger-Gilbert silt loam	19.07	October 6, 2017
Winnsboro, LA	May 22, 2018	Gigger-Gilbert silt loam	16.21	Failed

Inoculum Concentration Results Results from the four experiments were combined based on non-significant experiment x treatment interactions (P=0.6951). At 1 cc of inoculum/15 cm furrow, there was no significant emergence reduction, with a mean of 4.06 plants/pot compared to 5.13 plants/pot in non-treated pots (P=0.1121); however, there were significant (P<0.0001) reductions in emergence from 3.38 to 1.25 plants/pot at 2 to 10 cc inoculum/15 cm furrow, respectively (Figure 2.2). At 1 cc inoculum/15 cm furrow, there was a significant (P=<0.0001) 39.0 % shoot weight reduction. Plants inoculated at rates of 2 to 8 cc inoculum/15 cm furrow also had significant (P=<0.0001) 54.6-59.7 % shoot weight reductions, while a 74.8 % reduction occurred at the 10 cc/15 cm furrow rate (Figure 2.3). Root weights were significantly reduced by 56.9 % at 1 cc, 68.2-76.9 % at 2 to 8 cc/15 cm furrow, and 87.3 % at 10 cc/15 cm furrow (*P*=<0.0001) (Figure 2.4). Based on these data, we decided to use 1 cc/15 cm furrow for variety screening. We did not want significantly reduced emergence; however, we needed significant root and shoot weight reduction to challenge varieties and have enough remaining measurable tissue.

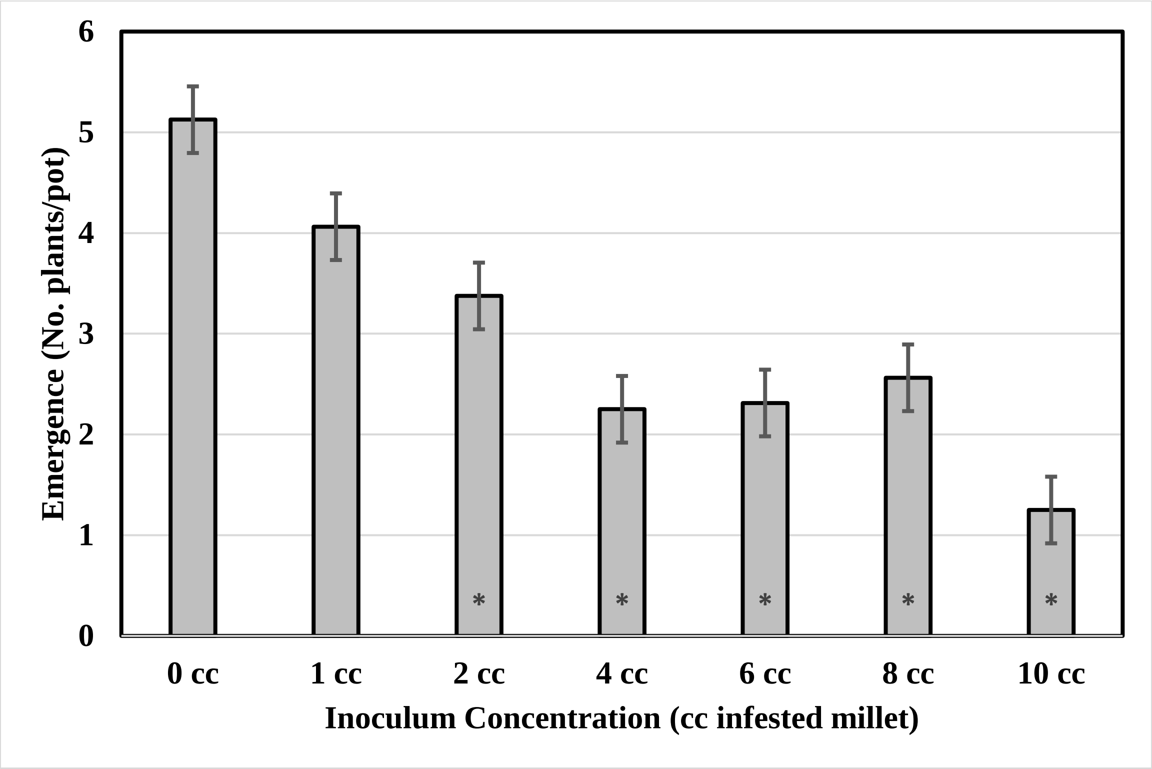

Figure 2.2. Emergence (No. plants/pot) 21 days after inoculation with *Xylaria* sp. at 1, 2, 4, 6, 8, or 10 cc/15 cm furrow of colonized Japanese millet.

*Denotes statistical difference according to Dunnett's means comparison to the non-inoculated control (P=0.05). Error bars represent the standard error of the mean.

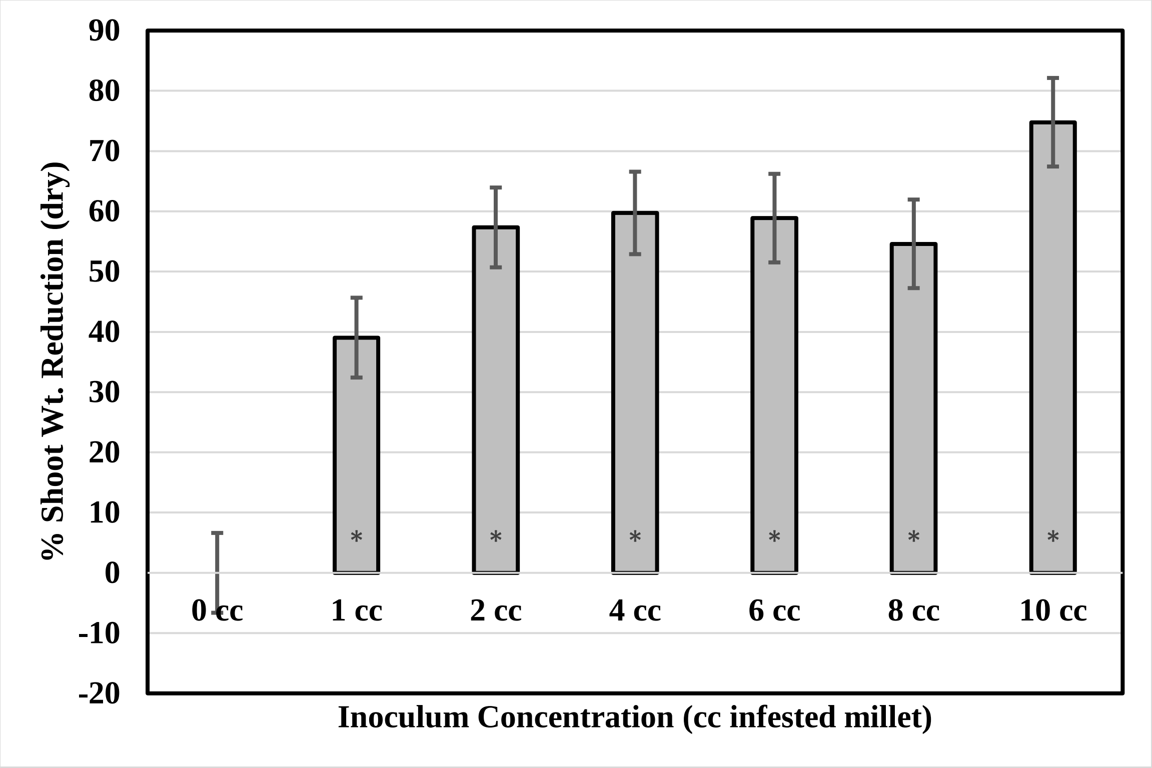

Figure 2.3 % Shoot wt. reduction (dry) 21 days after inoculation with *Xylaria* sp. at 1, 2, 4, 6, 8, or 10 cc/15 cm furrow of colonized Japanese millet.

*Denotes statistical difference according to Dunnett's means comparison to the non-inoculated control (P=0.05). Error bars represent the standard error of the mean.

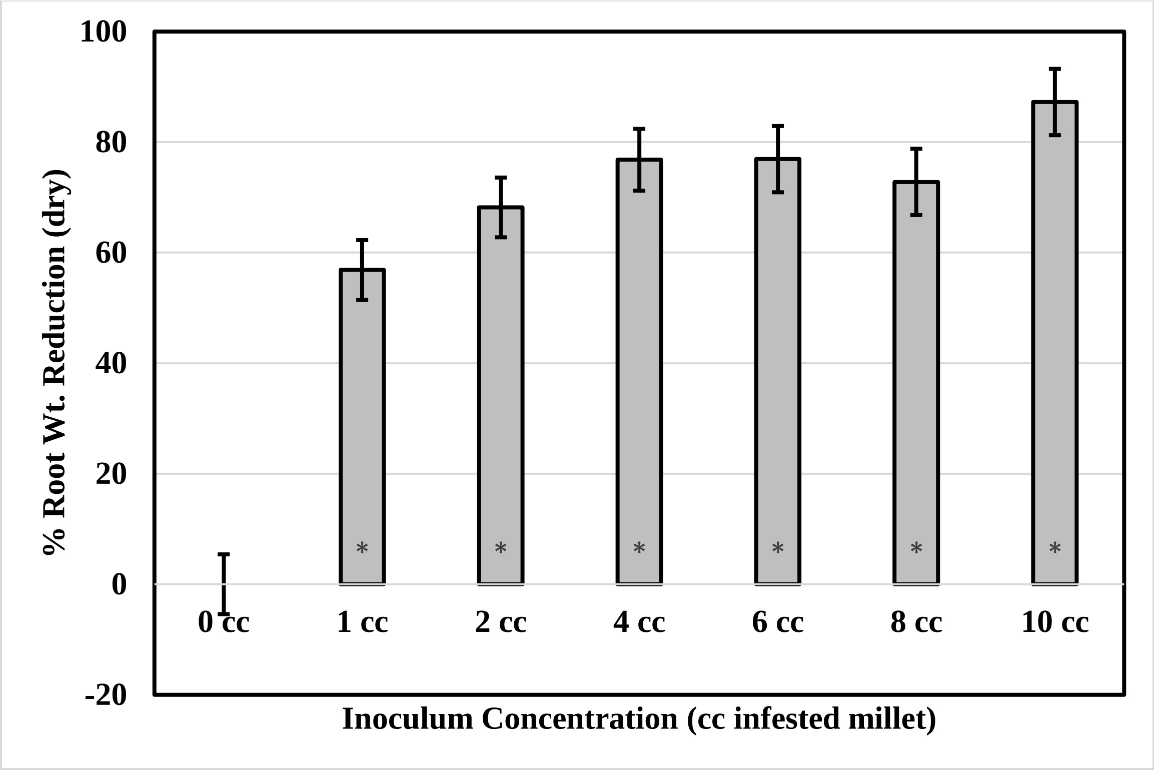

Figure 2.4. % Root wt. reduction (dry) 21 days after inoculation with *Xylaria* sp. at 1, 2, 4, 6, 8, or 10 cc/15 cm furrow of colonized Japanese millet.

*Denotes statistical difference according to Dunnett's means comparison to the non-inoculated control (P=0.05). Error bars represent the standard error of the mean.

Variety Screening Results There were significant reductions in emergence, root dry weight, and shoot dry weight. Seed germination percentage, seed treatments, and fungicide efficacy were unknown factors. Shoot weight is a function of root weight, and taproot decline initially affects the roots; therefore, root dry weight was the most reliable parameter used to determine the degree of susceptibility. Based on paired t-tests, there were significant reductions in root dry weight in 97 varieties ranging from 48 to 85% (Table 2.3). Varieties (n=97) with root weight reductions of ≥48% were considered susceptible (Table 2.3). Varieties (n=25) with a reduction ranging from 36-48% were considered moderately susceptible (Table 2.4), varieties (n=16) ranging from 24-36% reduction were considered moderately resistant (Table 2.5), and varieties (n=7) with <24% were considered resistant (Table 2.6). Means of the susceptible and resistant varieties were 61 and 19 % root weight reduction, respectively (Table 2.3, Table 2.6).

Table 2.3. Commercial soybean varieties from the 2016 LSU AgCenter OVT considered **susceptible** to *Xylaria* sp. as determined by artificial inoculation in the greenhouse.

Variety	**% Root Wt. Reduction**	**Variety**	**% Root Wt. Reduction**
S52LL66	85	UA 5414RR	60
5461 LL	84	4790 RR2	60
CZ4540LL	82	S39-C4	59
AG 44X6	78	P47T36R	59
DGX4845RR2	78	Rev 48A26	59
AG 47X6	77	GS47R216	59
GS45R216	76	Go Soy 5515LL	58
CZ 4898RY	74	S45W9	58
REV 47R34	73	UA 5814HP	57
S12-3782	73	CZ 3841LL	57
P5414LLS	72	Armor 50-D04	57
5170 RR2	72	Go Soy 5214GTS	57
CZ 5445LL	71	R09-430	57
Rev 48L63	71	5N452R2	56
Armor 47-R70	70	4670RR2	56
S12-4718	70	CZ 3991RY	54
CZ 5242LL	70	S12-3791	54
P4613RY	69	AG 53X6	54
S56RY84	69	REV 52A95	54
CZ 5150LL	69	R10-197RY	54
5N523R2	68	AG59X7	54
P4757RY	68	S42-P6	53

(table cont’d)

Variety	% Root Wt. Reduction
Armor 49-D66	67
Go Soy LELAND	67
Armor 49-D90	66
5N424R2	66
P4247LL	66
Schillinger 557.RC	66
Go Soy 4913LL	66
4825 RR2	65
CZ 5147LL	65
Go Soy 4714GTS	65
ARX5506	65
P47T89R	65
S39-T3	64
ARX4706	64
R10-230	64
P4900RY	64
S48XT56	62
S57RY26	62
CZ 4656RY	62
Rev 45A46	61
P5226RYS	61
CZ 5515LL	61
4587 LL	61
AG 49X6	61
CZ 4590RY	61
UA 5213C	61
REV 49A75	61
P4211RY	53
CZ 4748LL	52
UA 5014C	52
S48RS53	52
Schillinger 5220.RC	52
Armor 47-D17	51
CZ 3945LL	51
P5289RY	51
AG 46X7	51
4970 RR	51
Go Soy 4915R2	51
5N414R2	51
Go Soy 49G16	50
CZ 4959RY	49
5230 RR2	49
P5555RY	49
S45XS66	49
S11-20124	49
CZ 4181RY	49
AG 54X6	49
Rev 48A76	48
AG 55X7	48
P4930LL	48
S55Q3	48
5520RR2	48
CZ 4044LL	48
MEAN	**61**

Table 2.4. Commercial soybean varieties from the 2016 LSU AgCenter OVT considered **moderately susceptible** to *Xylaria* sp. as determined by artificial inoculation in the greenhouse.

Variety	**% Root Wt. Reduction**	**Variety**	**% Root Wt. Reduction**
P4588RY	48	S11-17025	44
5N406R2	47	P41T33R	42
5N480R2	47	Rev 51A56	41
S43RY95	46	S52RY77	41
Rev 49L49	46	S12-2418	40
Rev 49R94	46	P54T94R	40
Rev 56R63	46	S49LL34	39
Armor 48-D80	45	CZ 4222LL	38
Go Soy 5115LL	44	Rev 57R21	37
Armor 48-D24	44	GS48R216	37
Armor 46-D80	44	CZ 4105LL	37
S47-K5	44	P4814LLS	37
4967 LL	44	**MEAN**	**43**

Table 2.5. Commercial soybean varieties from 2016 LSU AgCenter OVT considered **moderately resistant** to *Xylaria* sp. as determined by artificial inoculation in the greenhouse.

Variety	**% Root Wt. Reduction**	**Variety**	**% Root Wt. Reduction**
S49XT07	35	CZ 5225LL	29
5625 RR2	35	P5725RY	28
S47RY13	35	4880 RR	28
AG 46X6	35	HBKLL4953	27
P4788RY	32	CZ 5375RY	26
AG 48X7	31	47016R	26
4995 RR	31	Armor 55-R68	25
Go Soy IREANE	31	**MEAN**	**30**
ARX4906	30		

Table 2.6. Commercial soybean varieties from 2016 LSU AgCenter OVT considered **resistant** to *Xylaria* sp. as determined by artificial inoculation in the greenhouse.

Variety	**% Root Wt. Reduction**
R07-6614RR	23
5067 LL	23
5N433R2	22
S42RY77	21
5N490R2	19
CZ 4818LL	19
OSAGE	8
MEAN	**19**

Field Confirmation Results During 2016, stands were numerically lower on all inoculated rows with the exceptions of CZ5225LL (moderately resistant in greenhouse tests) and REV57R21 (moderately susceptible in greenhouse tests), and significant ($\alpha=0.05$) reductions in stand occurred with 7 of 32 varieties (Table 2.7). The average stand reduction was 10.92 plants/20 ft. In the 8 varieties with significant differences in stand, the inoculated row averaged 67.46 plants/20 row ft and the non-inoculated row averaged 86.29 plants/20 row ft. In the 24 varieties with no significant differences the inoculated averaged 75.54 plants/20 row ft and the non-inoculated averaged 83.98 plants/row ft. Taproot decline incidence was increased in all inoculated rows except ARX5506 (susceptible in greenhouse tests) and was significantly higher in 20 of 32 varieties (Table 2.8) with an average of 8.70 % incidence. Mortality due to TRD was increased in all varieties except P4588RY and UA5814HP, with statistically significant increases in 4 of 32 varieties (Table 2.8) with an average increase in mortality of 1.05 % with inoculation. Plant height was lower in all inoculated varieties except S12-4718 and S48XT56, with significant height reduction in 9 out of 32 varieties (Table 2.9) and an average reduction of 2.74 inches. In the 9 varieties with a significant difference among heights, the inoculated plots averaged 31.61 in and the non-inoculated plots average height was 33.11 in. In the 23 varieties with no significant differences, average height in the inoculated plots was 35.94 in and non-inoculated average height was 37.9 in. Yields from MRRS during 2016 were numerically lower in all inoculated plots (Table 2.9). According to paired t-tests for each variety, yield in 7 of 32 varieties was significantly reduced by inoculation at MRRS during 2016 (Table 2.9). In the 8 varieties with significant yield differences, the inoculated plots averaged 10.26 bu/a and the non-inoculated plots average yield was 27.84 bu/a. In the 24 varieties without significant differences, the average yield was 16.18 bu/a in the inoculated plots and 25.49 bu/a in the non-inoculated plots.

Table 2.7. Stand (No. plants/20 row ft) of 32 varieties either inoculated (2cc/row ft) or not with Japanese millet infested with *Xylaria* sp. at planting in 2016 near Winnsboro, LA.

Variety	2016 Stand (Inoculated)	2016 Stand (Non-inoculated)	P-Value
4587 LL	52.25	81.75	0.0011
4970 RR	90.75	92.25	0.8043
5461 LL	78.50	87.25	0.5081
5N490R2	83.75	93.50	0.3573
Armor 46-D08	85.25	101.00	0.2111
Armor 47-R70	77.25	85.75	0.0245
ARX4906	85.75	89.50	0.6392
ARX5506	70.25	83.25	0.0336
CZ 5150LL	67.00	83.00	0.1535
CZ 5225LL	78.00	72.00	0.1612
CZ 5445LL	81.25	93.25	0.1449
Go Soy 5115LL	31.75	44.75	0.1261
Go Soy IREANE	81.00	93.50	0.0893
GS45R216	28.00	35.25	0.4422
P4588RY	90.50	98.75	0.1152
P47T89R	70.25	93.50	0.0207

(table cont’d)

Variety	2016 Stand (Inoculated)	2016 Stand (Non-inoculated)	P-Value
P4930LL	59.00	76.00	0.0346
P5289RY	75.75	79.00	0.6562
P5555RY	75.00	88.00	0.1638
R09-430	71.75	73.50	0.5436
R10-230	70.75	91.25	0.0514
Rev 48A26	92.75	97.00	0.4762
Rev 48A76	77.75	89.75	0.1474
REV 57R21	90.50	90.00	0.9335
S12-4718	55.67	79.00	0.1544
S47-K5	83.75	98.75	0.0590
S48XT56	78.75	82.75	0.5363
S52LL66	86.50	89.75	0.7488
S57RY26	68.25	86.50	0.0049
Schillinger 5220.RC	75.00	97.25	0.0122
UA 5414RR	67.25	70.75	0.2890
UA 5814HP	80.75	86.00	0.4852
MEAN	**73.77**	**84.48**	--

*Gray highlighting indicates statistically significant difference according to paired t-tests (α=0.05).

Table 2.8. Incidence (%) and mortality (%) (No. plants/20 row ft) of 32 soybean varieties either inoculated or not with sterilized Japanese millet infested with *Xylaria* sp. in 2016 near Winnsboro, LA.

Variety	TRD Incidence (Inoculated)	TRD Incidence (Non-Inoculated)	P-Value	Mortality (Inoculated)	Mortality (Non-inoculated)	P-Value
4587 LL	7.50	1.75	0.0012	3.00	0.00	0.0349
4970 RR	21.25	4.50	0.0109	3.75	0.25	0.0689
5461 LL	15.50	2.00	0.0158	0.75	0.50	0.7888
5N490R2	14.75	1.50	0.0368	2.00	0.00	0.1162
Armor 46-D08	14.00	3.00	0.0029	1.50	0.00	0.1027
Armor 47-R70	17.25	4.75	0.0586	1.00	0.25	0.3189
ARX4906	16.75	4.50	0.0005	2.50	0.25	0.1354
ARX5506	7.75	9.50	0.7210	1.00	0.50	0.4950
CZ 5150LL	10.00	9.00	0.8498	1.25	0.75	0.1817
CZ 5225LL	13.25	4.25	0.0107	1.00	0.50	0.3910
CZ 5445LL	10.00	5.00	0.3140	1.75	0.00	0.2126

(table cont'd)

Variety	TRD Incidence (Inoculated)	TRD Incidence (Non-Inoculated)	P-Value	Mortality (Inoculated)	Mortality (Non-inoculated)	P-Value
Go Soy 5115LL	4.50	2.25	0.2545	0.75	0.25	0.3910
Go Soy IREANE	12.50	1.75	0.0446	0.50	0.00	0.1817
GS45R216	6.00	2.00	0.0469	1.00	0.00	0.1817
P4588RY	13.00	3.75	0.0761	0.00	0.50	0.3910
P47T89R	10.50	5.50	0.1654	2.00	0.00	0.0163
P4930LL	11.25	5.50	0.0556	1.00	0.50	0.1817
P5289RY	12.75	4.50	0.0440	1.75	0.00	0.0060
P5555RY	12.00	5.00	0.1253	0.25	0.00	0.3910
R09-430	11.75	1.75	0.0004	0.75	0.25	0.1817
R10-230	14.00	5.25	0.0238	0.50	0.00	0.1817
Rev 48A26	18.50	3.25	0.0088	1.00	0.50	0.1817
Rev 48A76	16.00	1.25	0.0479	1.25	0.00	0.0796
REV 57R21	11.25	3.00	0.0380	1.50	0.25	0.2783
S12-4718	7.00	3.50	0.2066	0.50	0.00	0.3910
S47-K5	11.75	3.00	0.0622	0.75	0.25	0.4950
S48XT56	8.50	4.25	0.2200	0.75	0.25	0.3910
S52LL66	14.00	3.00	0.0098	0.25	0.25	1.0000
S57RY26	10.50	0.75	0.0178	1.25	0.00	0.1942
Schillinger 5220.RC	11.75	3.00	0.0263	2.50	0.00	0.0154
UA 5414RR	13.75	2.50	0.0204	1.25	0.00	0.0796
UA 5814HP	13.25	1.25	0.0052	0.25	0.50	0.3910

*Gray highlighting indicates statistically significant difference according to paired t-tests (α=0.05).

Table 2.9. Height (inches) and yield (bu/A) of 32 soybean varieties either inoculated or not with *Xylaria* sp. in 2016 near Winnsboro, LA.

Variety	Height (Inoculated)	Height (Non-inoculated)	P-value	Yield (Inoculated)	Yield (Non-Inoculated)	P-value
4587 LL	29.70	36.00	0.0006	10.77	29.67	0.0196
4970 RR	38.60	40.15	0.2339	16.73	30.70	0.0818
5461 LL	38.63	38.88	0.7053	18.58	37.20	0.1161
5N490R2	35.75	38.53	0.1213	12.94	27.17	0.0708
Armor 46-D08	32.53	35.73	0.0172	11.19	27.88	0.0635

(table cont'd)

Variety	Height (Inoculated)	Height (Non-inoculated)	P-value	Yield (Inoculated)	Yield (Non-Inoculated)	P-value
Armor 47-R70	34.85	37.45	0.0332	7.88	28.35	0.0236
ARX4906	33.43	37.73	0.0109	19.60	34.22	0.0653
ARX5506	33.60	36.20	0.1822	15.59	18.46	0.6739
CZ 5150LL	35.50	37.38	0.3068	19.45	25.39	0.2666
CZ 5225LL	31.70	33.53	0.3099	18.70	25.07	0.2878
CZ 5445LL	37.73	38.40	0.3484	21.33	26.66	0.5538
Go Soy 5115LL	25.60	33.33	0.0017	10.09	26.41	0.0842
Go Soy IREANE	39.40	42.10	0.1496	13.59	16.19	0.6714
GS48R216	28.25	33.43	0.0823	9.89	31.18	0.0020
P4588RY	38.63	40.23	0.2113	7.36	25.20	0.0319
P47T89R	30.58	33.70	0.0123	13.15	30.19	0.0882
P4930LL	28.95	34.00	0.1239	9.93	21.08	0.1058
P5289RY	33.15	35.18	0.0524	13.66	21.98	0.0807
P5555RY	32.73	36.90	0.0413	19.41	19.54	0.9311
R09-430	39.25	40.10	0.3321	20.36	31.44	0.1231
R10-230	33.48	38.10	0.0407	20.25	26.57	0.3596
Rev 48A26	36.73	37.70	0.1237	11.53	23.99	0.1457
Rev 48A76	33.68	37.98	0.1009	13.75	31.68	0.0254
REV 57R21	35.95	37.10	0.3792	15.78	32.54	0.0976
S12-4718	32.50	31.18	0.9037	26.30	26.82	0.9605
S47-K5	41.55	42.25	0.5986	16.09	26.05	0.3886
S48XT56	38.23	37.58	0.7462	12.99	16.88	0.3462
S52LL66	34.73	38.10	0.0648	11.33	23.56	0.0486
S57RY26	42.35	43.45	0.5490	19.58	27.36	0.3139
Schillinger 5220.RC	31.55	36.08	0.0080	10.82	25.22	0.0153
UA 5414RR	35.45	38.98	0.0761	16.45	17.39	0.5646
UA 5814HP	36.23	39.28	0.0503	11.22	20.01	0.2391
MEAN	**34.72**	**37.40**	--	**14.88**	**26.00**	--

*Gray highlighting indicates statistically significant difference according to paired t-tests (α=0.05).

During 2017 at MRRS, stand was lower in all inoculated rows except those of CZ 5225LL and REV 57R21, with statistically significant reductions in 16 of 32 varieties (Table 2.10). The average stand reduction was 17.8 plants/20 row ft. Incidence of TRD was numerically higher in 23 varieties, significantly in one, AG54X6 (Table 2.11) with an average of 1.18 % incidence. Mortality due to TRD was numerically higher in 20 of 32 varieties (Table 2.11) with an average increase in mortality of 0.46 % with inoculation. Plant height was reduced in all inoculated rows except for four varieties, Armor 49-D66, ARX5506, CZ5242LL, and DGX4845RR2 (Table 2.12). Significant reductions in plant height occurred in 6 of 32 varieties (Table 2.12) with an average reduction of 2.64 inches. Inoculation with the TRD pathogen reduced yield in all varieties, significantly in 14 of the 32 varieties (Table 2.12).

Table 2.10. Stand (No. plants/20 row ft) of 32 varieties either inoculated (2 cc/row ft) or not with Japanese millet infested with *Xylaria* sp. at planting in 2017 near Winnsboro, LA.

Variety	**Stand (Inoculated)**	**Stand (Non-Inoculated)**	**P-value**
4880RR	55.25	66.33	0.0447
4995RR	50.67	73.50	0.0323
5067LL	48.75	63.00	0.0556
5170 RR2	47.00	62.50	0.2952
5461 LL	60.00	66.00	0.6900
5N433R2	54.25	72.00	0.1243
AG 46X7	58.67	75.67	0.1638
AG46X6	61.75	74.75	0.0045
AG53X6	49.00	69.50	0.0324
AG54X6	57.50	69.00	0.1487
Armor 49-D66	64.67	77.67	0.2716
Armor 49-D90	41.33	62.75	0.0108
ARX5506	54.00	72.00	0.1204
CZ 4818LL	60.33	66.75	0.0474
CZ 5147LL	42.25	91.00	0.1841
CZ 5242LL	58.00	70.67	---
CZ 5375 RY	38.67	62.75	0.0692
DGX4845RR2	53.25	70.00	0.2578
Go Soy 4913LL	48.00	73.75	0.0585
Go Soy 5214 GTS	54.33	74.33	0.4121
Go Soy Irene	51.00	61.50	0.5359
Go Soy Leland	49.00	62.25	0.0489

(table cont'd)

Variety	Stand (Inoculated)	Stand (Non-Inoculated)	P-value
GS48R216	53.75	71.50	0.0156
P5752 RY	30.00	56.67	0.0242
R10-197RY	44.50	56.67	0.0189
47016R	57.67	75.00	0.0291
S12-3782	23.00	59.75	0.0024
S45W9	70.00	76.75	0.2665
S47RY13	43.33	67.00	0.0213
S48RS53	47.75	65.75	0.0558
S52LL66	56.50	73.33	0.0249
S56RY84	48.33	63.50	0.0341
MEAN	**51.02**	**68.86**	**--**

*Gray highlighting indicates statistically significant difference according to paired t-tests (α=0.05).

Table 2.11. Incidence (%) and mortality (%) (No. plants/20 row ft) of 32 soybean varieties either inoculated (2cc/row ft) or not with sterilized Japanese millet infested with *Xylaria* sp. in 2017 near Winnsboro, LA.

Variety	Incidence (Inoculated)	Incidence (Non-Inoculated)	P-value	Mortality (Inoculated)	Mortality (Non-Inoculated)	P-value
4880RR	2.50	1.33	0.2999	1.50	0.00	0.2697
4995RR	2.67	1.75	0.1835	0.67	0.75	0.6667
5067LL	1.25	1.75	0.6376	0.75	0.00	0.2152
5170 RR2	2.00	1.50	0.5000	0.75	0.00	0.5000
5461 LL	3.67	3.33	0.7592	2.00	1.33	0.7048
5N433R2	2.50	2.50	1.0000	0.50	0.50	1.0000
AG 46X7	0.33	1.33	0.2048	2.00	1.67	0.7952
AG46X6	1.75	1.25	0.4950	1.25	0.50	0.5472
AG53X6	2.67	1.75	0.4226	1.00	1.75	0.5471
AG54X6	5.00	2.00	0.0099	1.25	1.33	0.8399
Armor 49-D66	2.33	1.33	1.0000	1.33	1.00	0.5000
Armor 49-D90	4.00	1.00	0.2254	1.33	1.00	0.1835
ARX5506	3.33	4.33	0.7952	0.33	0.33	1.0000
CZ 4818LL	5.67	2.75	0.3468	2.67	0.75	0.0742
CZ 5147LL	3.00	2.00	0.2522	0.75	0.75	1.0000
CZ 5242LL	0.00	0.00	.	1.00	0.25	0.5000
CZ 5375 RY	1.67	2.50	0.4778	1.00	1.00	1.0000
DGX4845RR2	2.75	0.33	0.2507	0.25	0.67	0.1835

(table cont'd)

Variety	Incidence (Inoculated)	Incidence (Non-Inoculated)	P-value	Mortality (Inoculated)	Mortality (Non-Inoculated)	P-value
Go Soy 4913LL	1.50	2.75	0.3416	0.50	1.25	0.4444
Go Soy 5214 GTS	3.00	1.33	0.6784	0.50	0.33	0.4226
Go Soy Irene	1.75	2.75	0.6301	0.50	0.25	0.3910
Go Soy Leland	2.50	2.25	0.5000	1.50	0.25	0.5000
GS48R216	6.00	3.50	0.3615	1.25	1.00	0.8088
P5752 RY	3.00	2.33	0.6254	0.25	0.67	0.4226
R10-197RY	2.75	1.33	0.3701	0.50	0.67	1.0000
47016R	4.67	1.50	0.3624	2.33	0.25	0.2254
S12-3782	1.67	1.50	0.6914	0.00	0.50	0.4226
S45W9	2.50	2.00	0.7177	0.75	0.25	0.4950
S47RY13	6.33	1.50	0.1994	1.33	0.00	0.4226
S48RS53	5.75	1.00	0.1826	0.75	0.25	0.3910
S52LL66	2.50	2.33	0.6349	0.75	0.33	0.7418
S56RY84	3.75	5.00	0.5158	0.00	1.75	0.1328

*Gray highlighting indicates statistically significant difference according to paired t-tests (α=0.05).

Table 2.12. Height (inches) and yield (bu/A) of 32 soybean varieties either inoculated or not with sterilized Japanese millet infested with *Xylaria* sp. in 2017 near Winnsboro, LA.

Variety	Height (Inoculated)	Height (Non-Inoculated)	P-value	Yield (Inoculated)	Yield (Non-Inoculated)	P-Value
4880RR	32.83	34.44	0.6254	29.97	52.59	0.0937
4995RR	24.33	32.25	0.0805	35.12	45.32	0.3744
5067LL	34.83	37.33	0.0055	26.60	50.00	0.0557
5170 RR2	34.58	36.25	0.0590	28.99	37.48	0.6354
5461 LL	34.78	36.67	0.4532	25.06	41.94	0.2151
5N433R2	35.92	36.83	0.7737	31.28	50.05	0.0352
AG 46X7	32.78	35.11	0.1257	21.91	57.60	0.0231
AG46X6	35.83	37.25	0.2942	27.12	51.53	0.0109
AG53X6	23.25	29.00	0.1043	25.36	49.77	0.0442
AG54X6	37.67	38.58	0.8859	21.70	46.54	0.1112
Armor 49-D66	31.92	31.75	0.9423	30.38	39.15	0.6129
Armor 49-D90	31.08	33.58	0.2475	26.74	51.25	0.0368
ARX5506	37.25	37.08	0.9517	29.64	50.32	0.3981

(table cont’d)

(table cont’d)

Variety	**Height (Inoculated)**	**Height (Non-Inoculated)**	**P-value**	**Yield (Inoculated)**	**Yield (Non-Inoculated)**	**P-Value**
CZ 4818LL	37.33	38.25	0.4773	31.00	50.26	0.0885
CZ 5147LL	22.25	25.00	0.2383	26.81	51.23	0.0161
CZ 5242LL	34.25	34.33	0.9198	28.98	45.97	0.3135
CZ 5375 RY	21.56	24.42	0.2526	24.17	54.50	0.0160
DGX4845RR2	31.75	30.67	0.6595	33.66	41.37	0.8467
Go Soy 4913LL	35.50	40.17	0.0354	29.77	54.59	0.0295
Go Soy 5214 GTS	26.08	35.08	0.1816	24.38	47.01	0.1246
Go Soy Irene	22.67	23.67	0.6247	30.12	50.65	0.0143
Go Soy Leland	23.33	27.92	0.1799	28.36	44.56	0.0014
GS48R216	29.75	33.08	0.0518	23.66	52.47	0.0132
P5752 RY	28.25	31.78	0.8857	22.51	53.13	0.0771
R10-197RY	24.92	26.22	0.3546	30.46	61.34	0.0556
47016R	31.33	34.58	0.0190	29.53	50.97	0.0577
S12-3782	34.00	37.58	0.1069	29.57	54.34	0.0106
S45W9	27.83	28.25	0.8108	31.57	59.39	0.1061
S47RY13	33.44	36.17	0.0110	20.36	55.34	0.0526
S48RS53	31.67	38.08	0.0082	27.15	54.76	0.0048
S52LL66	33.00	35.42	0.2191	28.34	43.09	0.2230
S56RY84	29.92	32.25	0.2923	25.45	55.03	0.0392
MEAN	**30.81**	**33.41**	**--**	**27.68**	**50.11**	**--**

*Gray highlighting indicates statistically significant difference according to paired t-tests (α=0.05).

Discussion Data from the greenhouse screening indicated that the TRD inoculum was effective and that soybean varieties varied in response to inoculation. Because of the wide range of root weight reductions, it is likely that commercial sources of TRD resistance exist, which could be a viable management option for producers. Our screening indicates 23 resistant or moderately resistant varieties that may be available to producers with a history of TRD on their farm. At the time of the screening, each of the varieties were commercially available; however, since soybean varieties are replaced so quickly, regular screening of varieties is needed to keep up with the marketplace. Seed treatments were unknown, likely varied among companies, and could have been a source of variation; however, in other ongoing studies, no promising seed treatments have been identified to date. Therefore, seed treatments likely had minimal effect.

Field confirmation of greenhouse results was important in this study because field-screening is less tedious, provides yield data, and is more representative of a real world situation. Based on field data, the inoculum appeared to be effective. Even though not all reductions were significant, most varieties showed trends towards reduced stand, height, and yield and increased incidence and mortality in inoculated plots. The ranges of reduced stands, heights, and yields in the field were greater in varieties that were deemed susceptible in the greenhouse than in varieties considered resistant or moderately resistant For the most part, varieties that had reduced stand, increased incidence and mortality, or reduced height and/or yield in the field were deemed susceptible in the greenhouse (Tables 2.13 & 2.14).

Of the 32 random varieties tested in the field in 2016, 29 of them showed significant differences in measured parameters between inoculated and non-inoculated. Only 1 of the varieties, 5N490R2, was described as resistant in the greenhouse. Three varieties: ARX 4906, CZ 5225LL, and Go Soy Ireane were described as moderately resistant in the greenhouse. We saw only an increased TRD incidence in the field for 5N490R2, CZ 5225LL, and Go Soy Ireane, which could be an indication that these cultivars have the ability to compensate for TRD infection. In the case of ARX 4906, which was deemed resistant in the greenhouse, we observed a significant increase in incidence and a significant decrease in plant height in response to inoculation in the field. There were no significant differences in other parameters, which may indicate that this variety also has the ability to compensate for stand loss and height reductions. The overwhelming majority (25 varieties) of field assessments agreed with greenhouse assessments during 2016.

Table 2.13. Soybean varieties showing significant stand reductions, incidence and mortality increases, and/or height and yield reductions in the field along with corresponding greenhouse screening designation during 2016.

Variety	**Significant Stand Reduction**	**Significant Incidence Increase**	**Significant Mortality Increase**	**Significant Height Reduction**	**Significant Yield Reduction**	**Greenhouse Designation**
4587 LL	X[1]	X	X	X	X	S[2]
Schillinger 5220.RC	X	X	X	X	X	S
Armor 47-R70	X			X	X	S
P47T89R	X		X	X		S
S57RY26	X	X				S
ARX 4906		X		X		MR
Armor 46-D08		X		X		MS
ARX 5506	X					S
P4930LL	X					S
Go Soy 5115LL				X		MS
GS48R216		X			X	S
4970RR		X				S
P5289RY		X	X			S
4970 RR		X				S
R10-230		X		X		S
Rev 48A76		X			X	S
5461 LL		X				S
5N490R2		X				R
S52LL66		X			X	S
CZ 5225LL		X				MR
Go Soy Ireane		X				MR
GS45R216		X				S
R09-430		X				S
R10-230		X				S
Rev 48A26		X				S
UA 5414RR		X				S

(table cont'd) (table cont'd)

Variety	Significant Stand Reduction	Significant Incidence Increase	Significant Mortality Increase	Significant Height Reduction	Significant Yield Reduction	Greenhouse Designation
UA 5414RR		X				S
UA 5814HP		X				S
P4588RY					X	MS
P5555RY				X		S

[1]"X" indicates significance according to paired t-tests (α=0.05) in the variety for the corresponding category.

[2]S=Susceptible, MS=Moderately Susceptible, MR=Moderately Resistant, R=Resistant.

In 2017, 24 varieties showed significance differences in at least one of the measured parameters when compared to the inoculated row. Five varieties that were deemed resistant or moderately resistant in the greenhouse had significant stand reductions only when inoculated in the field, indicating a possible tolerance. Two varieties classified as moderately resistant in the greenhouse, 47016R and S47RY13, had significant reductions in stand and height in the field. Even with the reduced stand and height, apparently these two varieties were able to compensate for *Xylaria* sp. infection. A significant height reduction was observed in 5067LL in the field, even with the greenhouse MR designation, which is indicative of an inoculum response without symptoms. Three varieties that were resistant or moderately resistant in the greenhouse had significantly lower yield in the field. Perhaps *Xylaria* sp. can affect soybean yield without producing foliar symptoms. During 2017, several problems were encountered at planting and throughout the season that introduced considerable variability, which may have confounded results. Other factors that may have increased variability in field trials include: natural infection, less-than-optimal stands, herbicide damage, or environmental stresses. Our greenhouse inoculum rate may be too high for the field, which may explain the response of greenhouse-determined resistant varieties. More testing is needed to further confirm our greenhouse results in the field.

Table 2.14. Soybean varieties showing significant stand reductions, incidence and mortality increases, and/or height and yield reductions in the field along with corresponding greenhouse screening designation during 2017.

Variety	**Significant Stand Reduction**	**Significant Incidence Increase**	**Significant Mortality Increase**	**Significant Height Reduction**	**Significant Yield Reduction**	**Greenhouse Designation**
4880RR	X[1]					MR[2]
4995RR	X			X		MR
AG46X6	X				X	MR
AG53X6	X				X	S
Armor 49-D90	X				X	S
CZ 4818LL	X					R
Go Soy Leland	X				X	S
GS48R216	X				X	MS
P5752 RY	X					MR
R10-197RY	X					S
47016R	X			X		MR
S12-3782	X				X	S
S47RY13	X			X		MR
S48RS53	X			X	X	S
S52LL66	X					S
S56RY84	X				X	S
AG54X6		X				S
5N433R2					X	R
5067LL				X		R
AG 46X7					X	S
CZ 5147LL					X	S
CZ 5375RY					X	MR
Go Soy 4913LL				X	X	S
Go Soy Ireane					X	MR

[1] "X" indicates significance in the variety for the corresponding category.

[2] S=Susceptible, MS=Moderately Susceptible, MR=Moderately Resistant, R=Resistant.

Although variable, there are strong indications that commercial sources of TRD resistance are available, and this knowledge base may provide producers with an important TRD management option. Breeders also may use this information to begin breeding for TRD resistance in the future. More variety evaluation and development should continue in the United States to keep up with a constantly evolving industry in the future.

CHAPTER 3. EFFECT OF SEED TREATMENT AND IN-FURROW FUNGICIDES ON TAPROOT DECLINE

Introduction Questions often arise from growers, consultants, and industry regarding seed treatment or in-furrow fungicides for the reduction and control of TRD. Fungicide seed treatments, consisting of a fungicide treatment directly on the seed could be a convenient and economical solution. There are over 70 seed treatments and in-furrow fungicides available to producers containing over 15 modes of action and many more mixtures. The most common of these modes of action are FRAC Codes 4, 11, and 12. Since seed treatments are applied directly to the seed before planting, there is no added effort or time with this technology. Seed treatments can be costly but can be used on a field-by-field basis if there is a history of seedling disease issues or if the area has been planted to soybeans for many years. In-furrow sprays are more complicated due to equipment, calibrations, and mixes, but, if effective, may be more efficacious than seed treatments and economically beneficial. Seed treatments and in-furrow sprays have previously been shown to be beneficial in soybean (Gaspar et. al. 2016) (Guy et. al. 1989) (Vosberg et. al. 2017).

Seed Treatment/In-Furrow Fungicide Efficacy Trials Seed treatment and in-furrow fungicide efficacy trials were conducted from 2016-2018 at MRRS, DREC, and UA, Rohwer (Table 1). A TRD susceptible variety, AsGrow 4632, was either treated in the laboratory or had a fungicide applied in the furrow at planting (Table 3.2). Plots consisted of 4 rows, 35 feet long with row 1 and 2 inoculated with 2cc/row ft infested Japanese millet as described in Chapter 2. These trials were conducted in a randomized complete block design with four replicates. Parameters measured included: emergence, incidence (%), mortality (%), plant height (inches), and yield (bu/a). Data were subjected to analysis of variance using JMP Pro 14 (SAS, Cary, NC, USA). Means were compared post hoc using Tukey's Honest Significant Difference Test

(P=0.05). Due to drought, misapplication of herbicides, excessive insect damage, and/or severe weather events, acceptable data was only achieved in four trials.

Table 3.1. Agronomic milestones for seed treatment and in-furrow fungicide field testing locations during 2016-2018 in Arkansas, Louisiana, and Mississippi.

Location	**Planting Date**	**Soil Type**	**Rainfall (inches)**	**Harvest Date**
UA, Rohwer	June 13, 2017	Herbert silt loam	2.99	October 10, 2017
UA, Rohwer	May 4, 2018	Herbert silt loam	19.05	September 19, 2018
MRRS	April 26, 2016	Gigger-Gilbert silt loam	20.49	September 2, 2016
MRRS	May 17, 2017	Gigger-Gilbert silt loam	19.07	September, 7, 2017
MRRS	May 1, 2018	Gigger-Gilbert silt loam	16.21	September 10, 2018
DREC	June 21, 2016	Bosket very fine sandy loam	17.6	October 26,2016
DREC	June 29, 2017	Bosket very fine sandy loam	18.41	November 16, 2017
DREC	April 25, 2018	Bosket very fine sandy loam	20.57	September 21, 2018

Table 3.2. Treatments, rates, and mode of actions used in fungicide seed treatment and in-furrow trials at the Macon Ridge Research Station, Delta Research and Extension Center and University of Arkansas, Rohwer during 2016-2018.

Treatment	**Rate**		**Mode of Action**	**Treatment Type**^{3}
Non-treated				
Vibrance	0.16	FL OZ/Cwt	SDHI	ST
Acquire	0.75	FL OZ/Cwt	Group 4	ST
Stamina	1.5	FL OZ/Cwt	QOI	ST
Vortex	0.15	FL OZ/Cwt	DMI	ST
Sercadis	4.4	FL OZ/A	SDHI	IF
Ridomil	3.7	FL OZ/A	Group 4	IF
Headline	10.8	FL OZ/A	QOI	IF
Topguard Terra	8	FL OZ/A	DMI	IF

(table cont’d)

Treatment	**Rate**		**Mode of Action**	**Treatment Type**[3]
Ilevo[1]	2	FL OZ/Cwt	SDHI	ST
Mertect[2]	0.64	FL OZ/Cwt	Group 1	ST
Topsin[2]	20	FL OZ/A	MBC	IF

[1] Ilevo was added for the 2017 season.

[2] Mertect and Topsin were added for the 2018 season.

[3] ST=seed treatment, IF=in-furrow

Results At the MRRS location in 2016, increased taproot decline incidence was observed in inoculated plots when compared with non-inoculated plots (Figure 3.1). In inoculated and non-inoculated plots, no seed treatments or in-furrow applications resulted in significantly lower disease incidence when compared to the non-treated control. In inoculated plots four treatments: Stamina (seed treatment), Ridomil, Headline, and Topguard Terra had significantly lower disease incidence when compared to seed treated with Acquire (Figure 3.1). There were no significant differences in yields among treatments (data not shown).

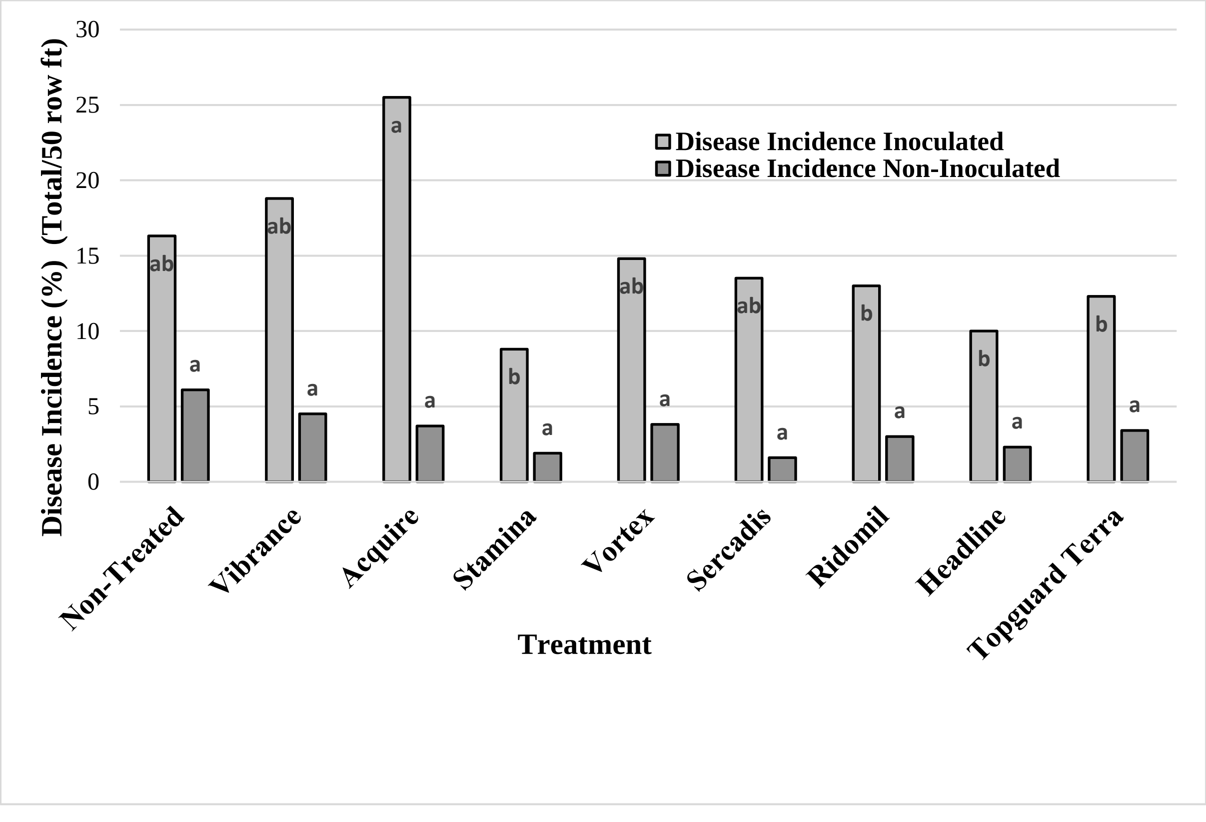

Figure 3.1. Effect of seed treatment and in-furrow fungicide application on soybean inoculated or non-inoculated with taproot decline of soybean, caused by *Xylaria* sp., in 2016 near Winnsboro, LA.

At the Arkansas location during 2017, the only parameter measured by the cooperator was yield. Overall, yields were significantly lower in inoculated plots (Figure 3.2). Yields in inoculated treatments trended lower in all treatments except Headline.

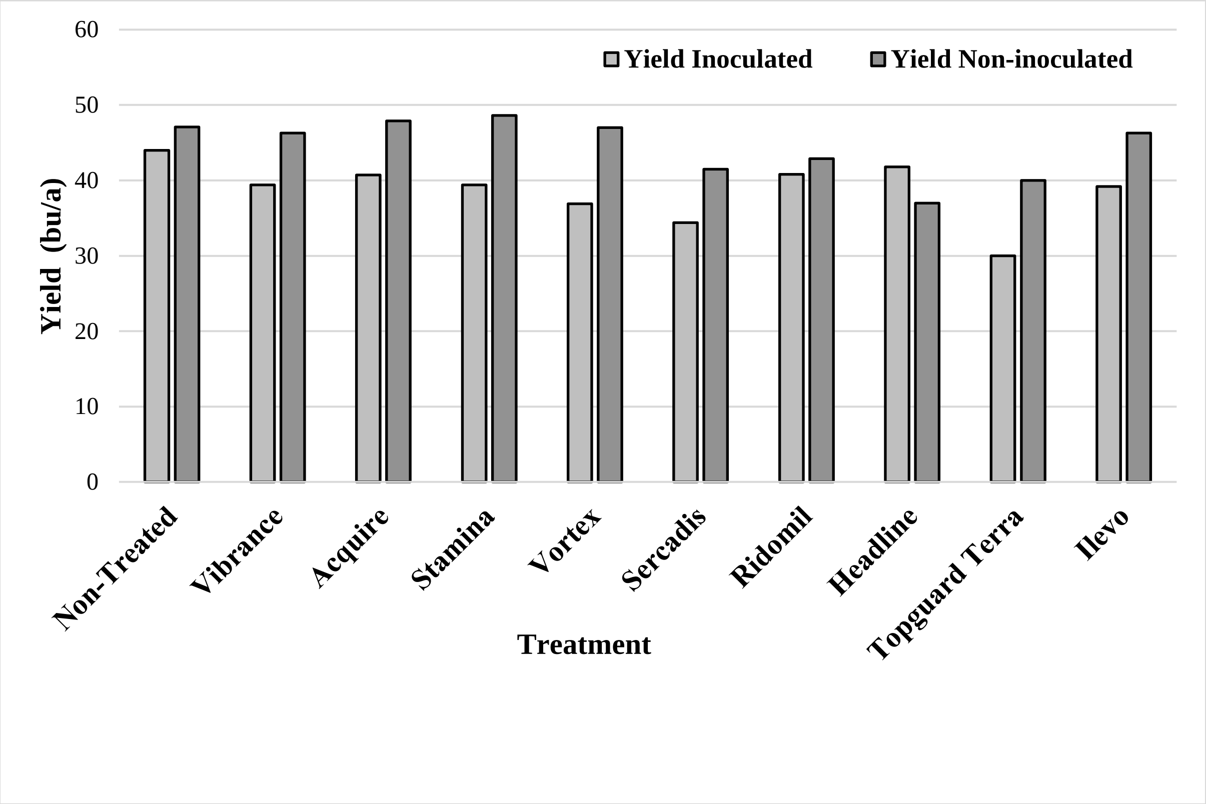

Figure 3.2. Effect of seed treatment and in-furrow fungicide application on yield of soybean inoculated with *Xylaria* sp., in 2017 in Rohwer, AR.

At the Arkansas location during 2018, data were not recorded for non-inoculated rows. Treatments with the highest stand counts included Stamina, Mertect, and Vortex with 140, 134, and 106 plants/20 row ft, respectively (Figure 3.3). There was a significant reduction in stand with the in-furrow treatment of Topguard Terra. There were no significant differences in yield among treatments (data not shown).

At the DREC location during 2018, there was significantly more yield preservation compared to all other treatments in inoculated plots treated with Ridomil, Headline, Topguard Terra and Topsin (Figure 3.4). Sercadis applied in-furrow resulted in significantly more yield preservation than Vibrance, Vortex, and Illevo, but less than Headline, Topguard Terra, and Topsin (Figure 3.4).

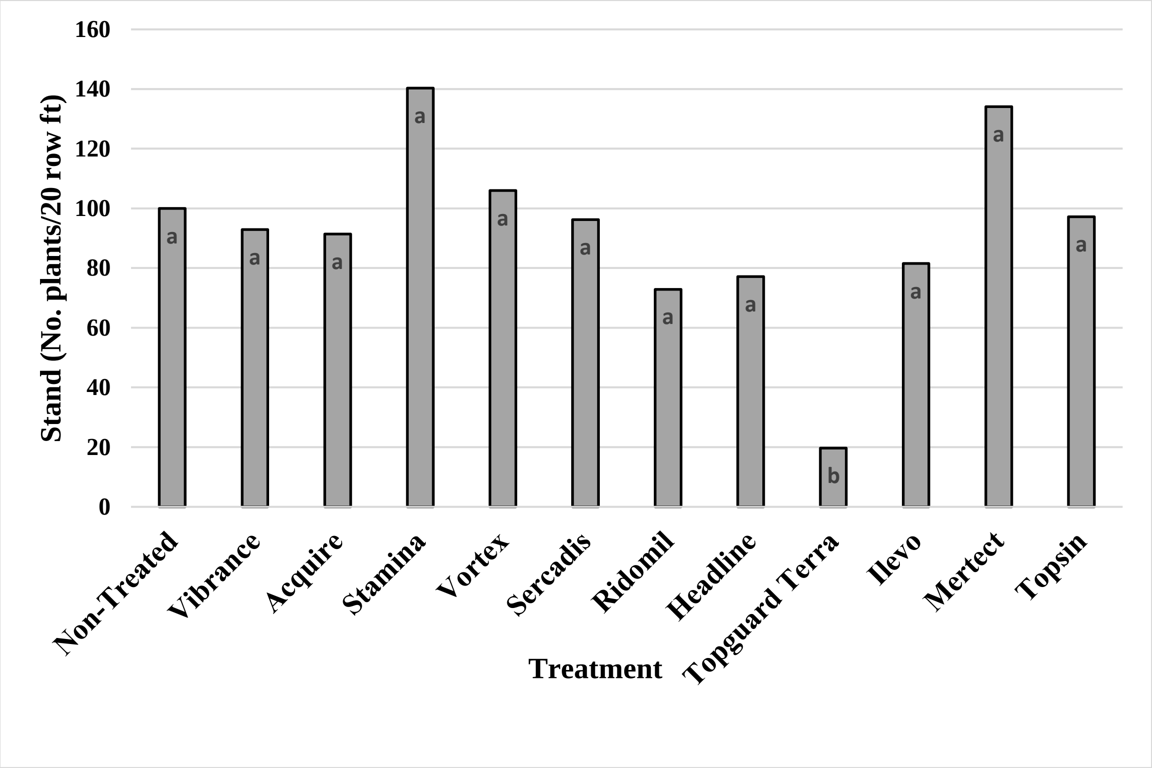

Figure 3.3. Effect of seed treatment and in-furrow fungicide application on stand in soybean inoculated with *Xylaria* sp. in 2018 in Rohwer, AR.

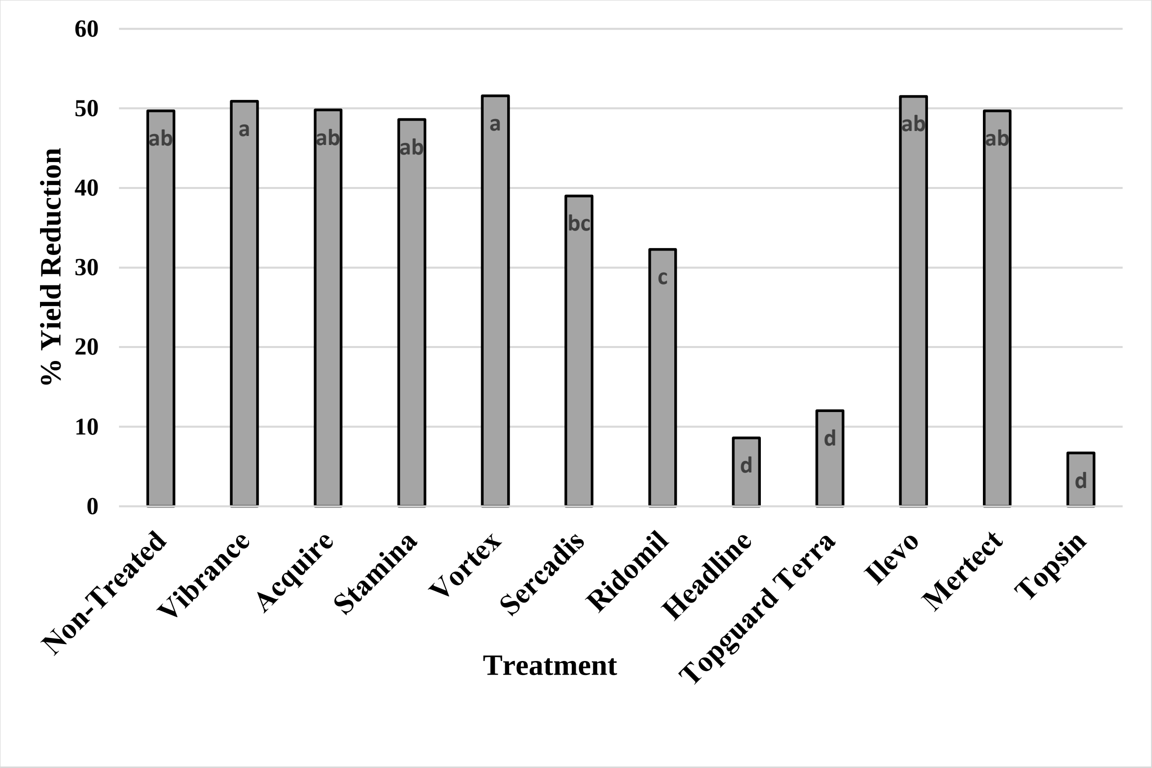

Figure 3.4. Effect of seed treatment and in-furrow spray on plots inoculated with *Xylaria* sp. in 2018 in Stoneville, MS.

Discussion Data from these trials indicate that the evaluated fungicide seed treatments are not effective at reducing taproot decline incidence or preserving yield, with the exception of TRD incidence trending downward with Stamina at MRRS during 2016. Interestingly, the in-furrow treatments with Headline, which contains the same fungicide as Stamina, seemed to be somewhat effective in two locations, particularly DREC during 2018. This could be indicative of more material delivered to the seed furrow with in-furrow applications compared to seed treatments. Additionally, Ridomil, Topguard Terra, and Topsin represent three separate modes of action and seem to be effective at reducing TRD incidence while preserving yield. Multiple, effective modes of action bode well for producer options in the marketplace and with resistance management. More research is needed in this area to further evaluate fungicide seed treatment and in-furrow application efficacy on taproot decline.

CHAPTER 4. A SOYBEAN TAPROOT DECLINE YIELD LOSS ESTIMATE

Introduction Yield loss studies have never been conducted on soybean affected by taproot decline (TRD). There are many diseases that can cause yield losses in soybean. These include nematodes (multiple species), frogeye leaf spot (*Cercospora sojina)*, Cercospora leaf blight (*Cercospora* spp.), seedling diseases (multiple species), and charcoal rot (*Macrophomina phaseolina*) (Allen et al. 2018). Each year state specialists provide yield loss estimates for soybean diseases using methods including field surveys, plant disease diagnostic clinic samples, variety trials, questionnaires to Cooperative Extension staff, research plots, grower demonstrations, crop consultant reports, foliar fungicide trials, and sentinel plot data (Allen et al. 2018). Yield losses define disease importance, since the bottom line of profit and resulting economic sustainability are most important to producers.

On-Farm Yield Loss Estimates On farm yield loss studies were conducted from 2015-2018. Crop consultants and producers identified fields with histories of taproot decline or ongoing epidemics. Upon thorough diagnosis and confirmation of the presence of TRD in a given field, ten locations per field were arbitrarily flagged, and the total number of

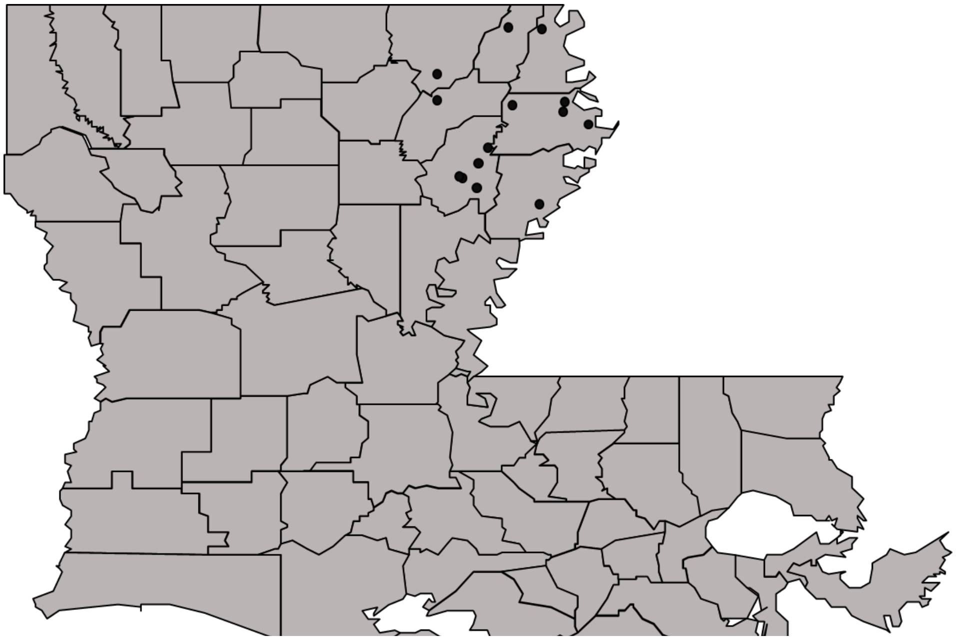

Figure 4.1. Map of locations of on-farm yield loss studies conducted from 2015-2018 in 7 parishes in Louisiana.

plants, TRD incidence, and TRD mortality was determined on 10 row feet per location. Prior to commercial harvest, plants were removed from the field, and yield was attained by threshing samples with a small plot combine (Kincaid, Haven, KS, USA). Yields were converted to bu/A. Yield loss estimations were conducted at 14 locations throughout northeast Louisiana during 4 years (Figure 4.1) (Table 4.1).

Table 4.1. Taproot decline on-farm yield loss trials and locations in northeast Louisiana during 2015-2018.

Location Name	Parish	Year	Soil Type
Bowie	West Carroll	2016	Grenada-Calhoun silt loams complex
E. Pond (MRRS)	Franklin	2018	Gigger-Gilbert silt loams complex
Evans	Madison	2018	Sharkey clay
Gilbert	Franklin	2015	Sharkey clay
Mound	Madison	2017	Sharkey clay
Oak Ridge	Morehouse	2017	Sterlington-Hebert silt loams complex
Rayville	Richland	2016	Rilla-Hebert silt loams complex
Tank	East Carroll	2017	Tensas Silty Clay
Truelove	Madison	2016	Dundee silt loam
Turtleback (MRRS)	Franklin	2018	Gigger-Gilbert silt loams complex
Vandeven	Tensas	2017	Sharkey clay
Vining	Madison	2018	Tensas-Sharkey clays complex
Williams	Franklin	2018	Dundee silty clay loam
Young	Franklin	2017	Sharkey clay

On Farm Yield Loss Estimation Results There was a significant and positive (P=0.10) correlation between % incidence and % mortality at 9 of 14 locations (Table 4.2). When combining data over these nine locations, TRD incidence and mortality were significantly and positively correlated (r = 0.736115, *P*=<0.0001) (Figure 4.2).

Table 4.2. Correlation of taproot decline % incidence and % mortality (P=0.10) from 14 on-farm locations in northeast Louisiana during 2015-2018.

Location	**% Incidence**	**% Mortality**	***P* Value**	**r Value**
Bowie	35.8	20.2	NS	--
Tank	10.2	7.5	<0.0001	0.9558
Oak Ridge	25.3	16.8	<0.0001	0.9399
Rayville	26.3	10.8	0.0135	0.6057
Truelove	38.7	14.5	0.0389	0.6073
Vining	4.3	5.0	0.0386	0.4800
Evans	7.7	6.0	NS	--
Mound	36.8	15.6	0.0010	0.7604
Williams	3.5	16.6	0.0362	0.4413
E. Pond (MRRS)	7.7	2.3	NS	--
Turtleback (MRRS)	4.5	1.3	NS	--
Gilbert	7.5	3.8	0.0002	0.8435
Young	2.3	1.9	<0.0001	0.9089
Vandeven	13.6	5.7	NS	--

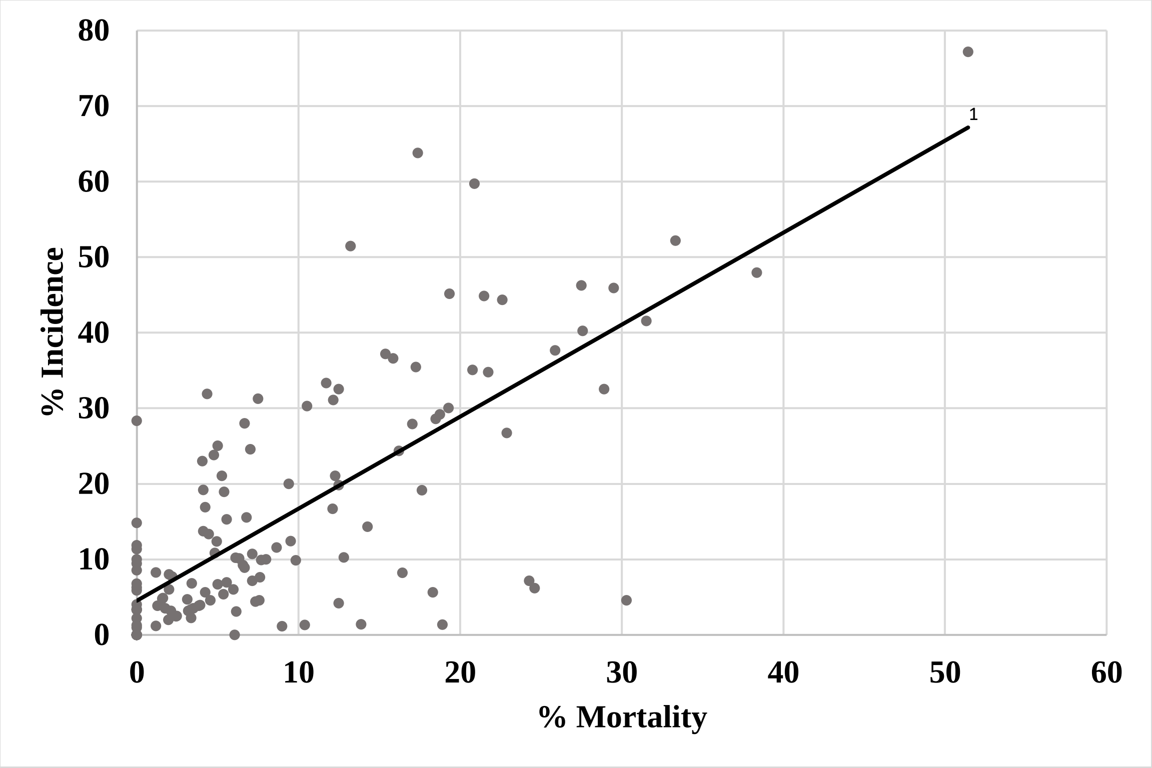

Figure 4.2. Relationship between soybean taproot decline incidence and plant mortality on 9 locations throughout northeast Louisiana from 2015-2018.

[1] Trend line equation=*% yield loss = 4.5310259 + 1.217929 * % incidence, r = 0.736115, *P*=<0.0001.

There were 7 locations where % incidence and % yield loss were significantly and positively correlated (Table 3). Over these seven locations, correlation of TRD incidence and yield loss due to TRD was significant and positive (r = 0.57205, *P*=<0.0001) (Figure 4.3).

Table 4.3. Correlation of taproot decline % incidence and % yield loss (P=0.10) from 14, on-farm locations in northeast Louisiana during 2015-2018

Location	% Incidence	% Yield Loss	*P* Value	r Value
Bowie	35.8	25.4	0.0803	0.3337
Tank	10.2	13.5	0.0247	0.4875
Oak Ridge	25.3	15.9	NS	--

(table cont'd)

Location	**% Incidence**	**% Yield Loss**	***P* Value**	**r Value**
Rayville	26.3	5.9	NS	--
Truelove	38.7	23.4	0.0074	0.7905
Vining	4.3	11.9	0.0086	0.6505
Evans	7.7	43.0	NS	--
Mound	36.8	10.4	0.0035	0.6756
Williams	3.5	12.7	NS	--
E. Pond (MRRS)	7.7	10.4	0.0256	0.4834
Turtleback (MRRS)	4.5	12.8	NS	--
Gilbert	7.5	10.5	0.0093	0.5914
Young	2.3	2.0	NS	--
Vandeven	13.6	4.7	NS	--

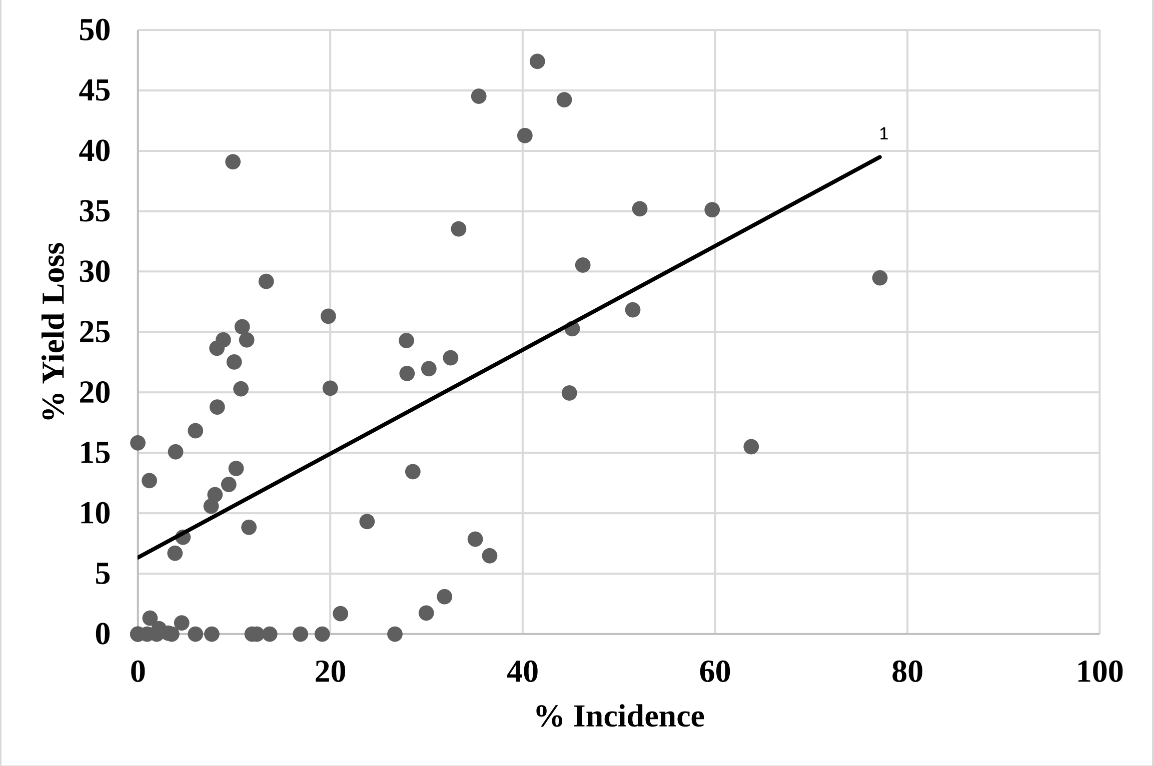

Figure 4.3. Relationship between soybean taproot decline incidence and yield loss over 7 locations throughout northeast Louisiana from 2015-2018.

[1] Trend line equation=*% yield loss = 6.326572 + 0.429816 * % incidence, r = 0.57205, *P*=<0.0001.

There also were 7 locations where % mortality and % yield loss were significantly and positively correlated (Table 4.4). Over these seven locations, mortality and yield losses caused by TRD were significantly and positively correlated (r = 0.60330, *P*=<0.0001).

Table 4.4. Correlation of taproot decline % mortality and % yield loss (P=0.10) from 14, on-farm locations in northeast Louisiana during 2015-2018.

Location	**% Mortality**	**% Yield Loss**	***P* Value**	**r Value**
Bowie	20.2	25.4	NS	--
Tank	7.5	13.5	0.0253	0.4846
Oak Ridge	16.8	15.9	NS	--
Rayville	10.8	5.9	0.0935	0.3497
Truelove	14.5	23.4	0.0342	0.6254
Vining	5.0	11.9	0.0583	0.4219
Evans	6.0	43.0	NS	--
Mound	15.6	10.4	0.0013	0.7463
Williams	16.6	12.7	0.0027	0.6965
E. Pond (MRRS)	2.3	10.4	NS	--
Turtleback (MRRS)	1.3	12.8	NS	--
Gilbert	3.8	10.5	0.0339	0.4494
Young	1.9	2.0	NS	--
Vandeven	5.7	4.7	NS	--

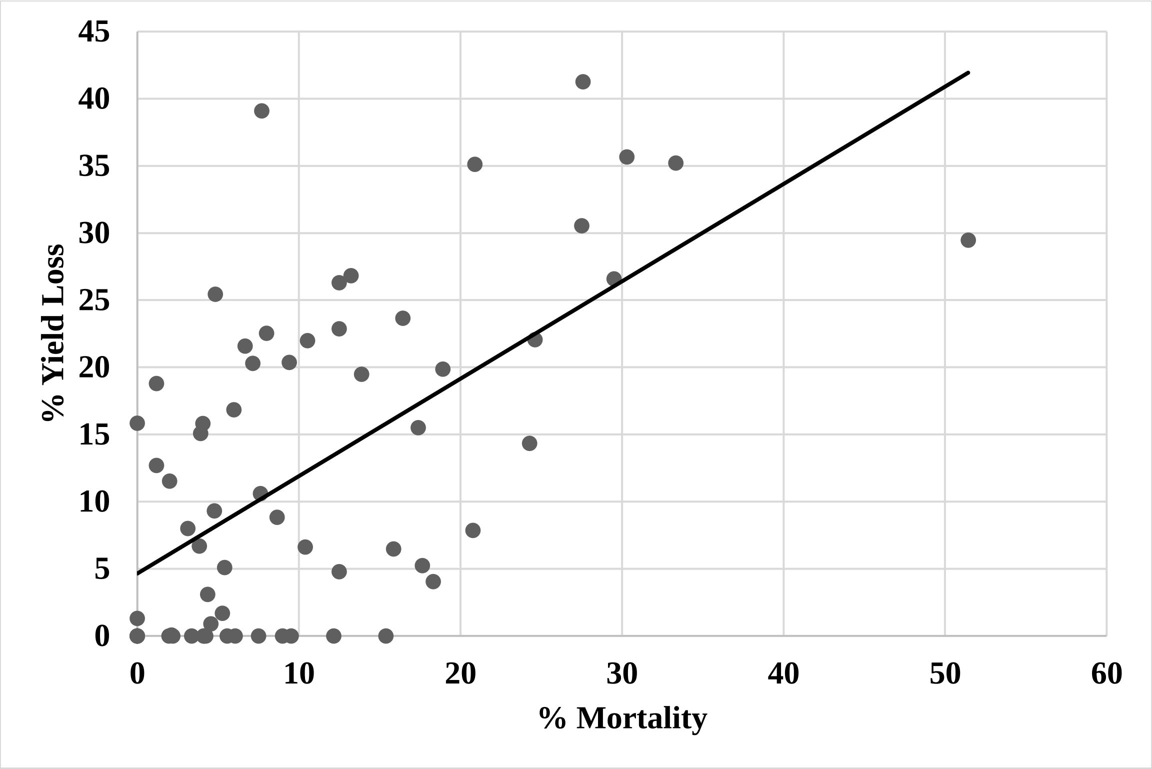

Figure 4.4. Relationship between soybean taproot decline yield loss and plant mortality over 7 locations throughout northeast Louisiana from 2015-2018.

[1]Trend line equation=*% yield loss = 4.6470625 + 0.7250549 * % mortality, r = 0.60330, *P*=<0.0001.

Discussion In the case of taproot decline, yield loss is assumed to be due to reduced photosynthesis, smaller plants and fewer pods, smaller seed and pod size, and total plant death, although loss mechanisms in leguminous crops are complicated and not yet fully understood (Yang et al. 1991). In our estimations incidence is positively correlated with mortality indicating that taproot decline progresses throughout the season causing death at multiple crop stages. Increased incidence can cause yield loss even without mortality due to less photosynthesis, smaller seed size, fewer pods per plant, reduced plant height, less nodes, and lodging. Increased mortality early during the growing season can result in lower than recommended plant populations affecting yield. Mortality during late reproductive stages results in incomplete pod fill and complete to partial yield loss for a given plant.

As demonstrated in previous chapters, genetic resistance is superior to any other compensation to yield loss by TRD. Taproot decline is an emerging and major issue in the southern United States. According to Allen et al. 2019, 2 million bushels of soybean were lost to the disease in the southern US, and this number should be expected to grow as disease incidence increases.

CHAPTER 5. SUMMARY

Soybean is produced in 16 states in the Southern US. Of these 16 states, the causal agent of taproot decline (TRD), *Xylaria* sp., has been found in Alabama, Arkansas, Louisiana, Mississippi, and Tennessee. It is believed that TRD reaches even farther north, however, this has not been confirmed. Distribution and genetic characterization of the pathogen is ongoing within the LSU Department of Plant Pathology and in cooperation with scientists in other southern states. In 2018, there were over 950 million bushels of soybean produced in the south. According to Allen et al. 2019, TRD accounted for over 2 million lost bushels of soybeans in Alabama, Arkansas, Louisiana, and Mississippi. In the 5 states where TRD has been confirmed, it is among the top five yield-limiting soilborne diseases, and in Louisiana ranks among the top 3 along with root-knot nematode and pod and stem blight.

These studies have described an effective inoculum production method, a variety screening process, and resistant varieties for producers. Although some of these varieties may not still be available, they can still be used in breeding programs as a basis for the next generation of TRD resistant soybeans. These studies also indicate that greenhouse screening is a valid way to test varieties against TRD during the off-season. Within about 3 weeks it can be determined if a variety is resistant or susceptible to TRD. Results from these studies also indicate that field inoculation and screening of soybean varieties is possible. We have identified fungicides that have potential activity against TRD, which may offer an additional management option for producers. Yield loss estimations have provided scientific evidence that TRD causes significant yield losses, which is important information for all stakeholders. Research on variety response, fungicide efficacy, and yield losses should continue providing stakeholders with key management information. Other future research efforts should include the effect of management practices such as crop rotation and tillage and on TRD.

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VITA

Myra Marie Arnold Purvis, daughter of David and Judith Arnold was born in 1981 in West Monroe, Louisiana. She is a lifelong resident of Louisiana, graduating from Franklin Academy in Winnsboro in 1999, and earning a Bachelor of Science in Agronomy from Louisiana Tech in 2003. She began working for the Dr. Rogers Leonard with the LSU AgCenter, Macon Ridge Research Station, upon graduation from Tech. In February of 2004, Dr. Boyd Padgett advertised a position of research associate for which Myra was selected. She has been with the AgCenter since that time. In 2013, Dr. Trey Price became the extension Plant Pathologist after Dr. Padgett took a management position. In 2014, Myra was accepted to the Plant Pathology graduate program at Louisiana State University under the direction of Dr. Trey Price. She currently resides in Delhi, Louisiana, is married to Jeremy, and has a son, David Allen. Myra is currently a MS candidate in Plant Pathology.