Effects of ILeVO® on soybean sudden death syndrome and soybean cyst nematode

by

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ABSTRACT

Fusarium virguliforme, the causal agent of soybean sudden death syndrome (SDS), and *Heterodera glycines*, soybean cyst nematode (SCN), are two of the most important pathogens of soybean. Host resistance is currently the main management strategy for both pathogens, and there are few other options available for each pathogen. Seed treatments are now an option for farmers for use of pesticides in early plant development. Bayer CropScience recently registered a seed treatment, ILeVO® (fluopyram), with reported activity against both SDS and SCN. The research described in the following manuscript tested ILeVO® in different combinations with currently available seed treatment products for management of each pathogen separately and together. All experiments were performed in temperature-controlled water baths in the greenhouse.

The first experiment evaluated seed treatments on soybean seedlings infected with SCN alone. Plants were grown for 30 days at 27˚C. The experiment was run three times and the data were combined for analysis. Results for this experiment were somewhat unclear. However, plants treated with a seed treatment combination of Trilex® + Allegiance® + Poncho/VOTiVO® + ILeVO® had less SCN females per gram of root when compared to the same combination without ILeVO®.

The second experiment examined the effects of ILeVO® on soybeans inoculated with *F. virguliforme* and *F. virguliforme* combined with SCN. For this experiment, water baths were maintained at 24˚C and plants were allowed to grow for 35 days. Three runs of this experiment were conducted, but the data from each run was analyzed separately due to changes in experimental design. No significant differences were found among the seed treatments in any of

the three runs for SDS foliar symptom severity. SDS root rot severity was significantly lower for Trilex® + Allegiance® + ILeVO® compared to Trilex® + Allegiance® in one of the three experimental runs. When looking at SDS and SCN in combination, there were significantly fewer SCN females per gram of root in the presence of ILeVO® for the contrast involving Trilex® + Allegiance® + Poncho/VOTiVO® and Trilex® + Allegiance® + Poncho/VOTiVO® + ILeVO® for the first run of the experiment. There were also significantly fewer SCN females per gram of wet root mass for the contrast of Trilex®+Allegiance®+ILeVO® compared to Trilex® + Allegiance® in the third run. These results indicate that ILeVO® may negatively affect SCN, but we detected no such negative effects of ILeVO® on SDS foliar disease symptoms in our greenhouse experiments.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Thesis Organization

This thesis is arranged into three chapters. Chapter one is the introduction and literature review. Chapter two covers the effects of ILeVO® on soybean cyst nematode (SCN). Finally, chapter three assesses the effects of ILeVO® on soybean sudden death syndrome (SDS) and the combination of SDS and SCN. Several side experiments were completed to adjust our experiments. The details and results for these side experiments are in the appendix.

Introduction and Literature Review

Soybean (*Glycines max*) is an important crop in the United States. In 2012, the United States produced over 3 billion bushels worth $43.1 billion ("SoyStats®", 2014). Disease is a yield-limiting factor and is a major challenge for farmers. Two very important soybean pathogens are *Fusarium virguliforme*, the causal organism of SDS, and *Heterodera glycines*, which is the SCN.

Every year SDS is a potential threat to soybean yield. In 2010, SDS caused an estimated 4.7 million metric tons yield loss nationwide (Bradley and Koenning, 2014). That year was particularly favorable for SDS and shows the type of impact SDS can have on soybeans (Leandro et al., 2010).

There are two ways that SDS can affect yield: foliar disease symptoms and root rot. The foliar phase is what characterizes the disease in the field. Foliar symptoms can be described as chlorosis of leaf tissue between the veins, which may later become necrotic. The foliar symptoms are also what cause yield loss. SDS foliar symptoms have been shown to have a negative correlation with yield (Hartman et al., 1995; Hershman et al., 1990; Luo et al., 2000; Scherm and

Yang 1996). The other aspect of SDS is the infection of soybean roots causing root rot. Luo et al. (2000) found that early root infection by *F. virguliforme* resulted in higher severity of foliar symptoms than did later infection. It is unclear, however, of the extent of yield loss associated with the root rot component of SDS. Other studies have shown that there is little to no correlation between root rot severity and foliar symptom severity (Njiti at al., 1997; Scherm and Yang, 1996). Regardless of the effects of root rot, SDS can be incredibly destructive.

Walters discovered SDS in Arkansas (Roy et al., 1997) in 1971. Aoki et al. (2003) classified the causal agent of SDS as *Fusarium virguliforme* in 2003. SDS quickly spread throughout soybean-growing states and can now be found in Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Mississippi, Missouri, Nebraska, Ontario, South Dakota, Tennessee, and Wisconsin (Anderson and Tenuta, 1998; Bernstein et al., 2007; Chilvers and Brown-Rytlewski, 2010; Jardine and Rupe, 1993; Kurle et al., 2003; Tande et al., 2014; Roy et al., 1989; Rupe, 1989; Yang and Rizvi, 1994; Ziems et al., 2006). Integrating multiple management strategies is needed because of the destructive nature and wide geographical range of this disease.

Currently, options for managing SDS are somewhat limited. One main option for management is resistant soybean cultivars (Leandro et al., 2013; Rupe et al.1991). Another management option is through cultural practices. One cultural practice that has been used to control SDS is rotation to a non-host crop. However, the common corn-soybean rotation is ineffective for controlling SDS (Westphal and Xing, 2011; Xing and Westphal, 2009). Rotations with crops other than corn can reduce SDS (Abdelsamad et al., 2011; Rupe et al., 1997). Another cultural practice for SDS management is tillage, but results are inconsistent. One study showed that SDS foliar symptom severity was lower in tilled plots (Wrather et al., 1995). However Seyb

et al. (2007) found that long-term no-till practices may be more effective in reducing SDS. Delayed planting is another cultural practice used to reduce SDS (De Bruin and Pedersen, 2008; Hershman et al., 1990; Wrather et al., 1995). Infection of the roots early in the growing season, under the right environmental conditions, can bring about the most severe cases of SDS later in the season. Gongora-Canul and Leandro (2011) reported that inoculating at planting produced the most severe foliar SDS symptoms compared to inoculating days to weeks later and that foliar symptoms were less severe at warmer temperatures at planting. Another thing to consider about delayed planting is the length of the growing season. According to De Bruin and Pedersen (2008), delaying planting can actually lower yield potential and in some cases may be worse than the loss from disease.

From 2009 through 2011, SCN caused an estimated 23 million metric tons of yield loss on soybeans (Bradley and Koenning, 2014). The effects that SCN have on crop yields are highly dependent on the initial nematode population density present in a field (Francl and Dropkin, 1986; Schmitt et al., 2004). SCN can be difficult to detect in a field. In many cases there will be a yield reduction without any visual symptoms (Wang et al., 2003). The nature of this disease makes management an ongoing challenge.

In 1954, SCN was first discovered in the United States (Wrather et al., 1984). Today, SCN can be found in most states where soybeans are grown (Tylka and Marett, 2014). Like SDS, management of this disease has consisted mostly of host resistance and cultural practices such as rotation to a non-host crop (Howard et al., 1998; Niblack, 2005; Schmitt, 1991; Wrather et al., 1984). According to Niblack (2005), effective rotation for SCN management should include three phases; the rotation of a non-host crop, the rotation of an SCN-resistant soybean cultivar, and finally rotation to a SCN resistant cultivar with a different source of resistance. Host

resistance for SCN is limited to a few sources of resistance; a majority of SCN-resistant cultivars are generated from the same source of resistance, PI 88788 (Tylka and Mullaney, 2013). Additionally, unlike SDS, there are also seed treatments that are being marketed for the control of SCN such as: VOTiVO® (*Bacillus firmus*, Bayer CropScience), Avicta® (abamectin, Syngenta), and Clariva® (*Pasteuria nishizawae*, Syngenta). These three seed treatments are all relatively new for SCN management.

There are also instances where SDS and SCN have been shown to interact when they co-inhabit a field (Donald et al., 1993; Faghihi et al., 2013; Gao et al., 2006; Giammaria et al., 2004; Giammaria and Rupe, 2006; Giammaria et al., 2007; McLean and Lawrence, 1995). Each pathogen causes significant yield loss, but together they have the potential to cause even greater losses. According to Roy et al. (1989), SDS foliar symptoms are more pronounced in the presence of SCN.

Currently one option for managing soybean disease is seed treatments. The practice of treating seed can be traced back as far as the 1700s when a British agronomist noticed that wheat seed that had been accidentally brined, or soaked in salt water, were more capable of repelling smut infection (Tull, 1733). In the late 1960s, the first systemic seed treatment was introduced thus expanding the use of seed treatments in their ability to protect not just seed, but young plants (CropLife Foundation, 2013). From 2001 to 2011, the percentage of soybeans treated with seed treatments increased from 5 to 18% (CropLife Foundation, 2013). As the adoption of seed treatments on soybean has increased, the number of available active ingredients has risen, allowing farmers to apply seed treatments to protect against several pests.

There are two types of seed treatment that can be used for pathogen control: contact and systemic. A contact fungicide adheres to the seed surface (Mueller et al., 2013). The chemical is

then subject to environmental conditions and will eventually be washed away. This type of application can control a pathogen for a limited time in the area the chemical is applied. A systemic product, on the other hand, gives a more persistent protection to the seed after it is planted. A systemic product is taken up into the plant tissue after planting and can protect against both soil-borne and foliar pathogens (Mueller et al., 2013). Like contact products, systemic products will not persist indefinitely; however, the duration in or on the plant will be longer as the chemical cannot be washed away like a contact product is. Since the use of a seed treatment is limited to one application at the beginning of the season, systemic seed treatments would be better for pathogens like SDS and SCN, since the product will be present in the plant tissue.

Because SDS infection occurs so soon after planting, seed treatments conceivably should manage SDS. However, there are currently no registered seed treatments that effectively reduce both root and foliar symptoms of SDS (Weems et al., 2011). There are several products available that control other fungi from the *Fusarium* genus (Munkvold and O'Mara, 2002).

A number of seed treatments have been registered for treatment of nematode management on various crops. Abamectin has activity on *Meloidogyne incognita* for tomato (Qiao et al., 2012), tobacco (Muzhandu et al., 2014) and cotton (Faske and Starr, 2007). *Bacillus firmus* is a possible biological control agent against nematodes. *Bacillus firmus* reduces the number of *Rotylenchulus reniformis*, the reniform nematode, on cotton (Castillo et al., 2013). *Bacillus firmus* was also shown to have an effect *in vitro* on SCN (Schrimsher et al., 2011), *Radopholus similis*, *Meloidogyne incognita* and *Ditylenchus dipsaci* (Mendoza et al., 2008).

Fluopyram is a succinate dehydrogenase inhibitor (SDHI) of fungi, which is a compound that inhibits fungal respiration (Avenot and Michailides, 2010). Fluopyram, along with boscalid and penthiopyrad, are SDHIs that have exhibited activity across a broad spectrum of fungi

(Avenot and Michailides, 2010). Fluopyram has been used against several diseases including Alternaria late blight of pistachio (Avenot et al. 2012) and powdery mildew and leaf spot on cherry (Proffer et al., 2013) as a foliar application. According to Musson et al. (2011), fluopyram is also a systemic fungicide in the roots. Due to the broad spectrum activity of SDHI fungicides, it is possible that they may have activity on other fungi.

ILeVO® (fluopyram, Bayer CropScience Co.) is a new seed treatment that should be available for soybean for the 2015 growing season. Due to weather conditions in 2010 causing high prevalence of SDS, Bayer CropScience was able to do some research regarding SDS regarding fluopyram. Under field conditions they found that plants treated with fluopyram or a fungicide insecticide base in combination with fluopyram greatly reduced SDS foliar symptoms when compared to control plants and those with just an insecticide-fungicide base (Mueller et al., 2011). Early testing revealed that this product also might exhibit activity on plant-parasitic nematodes such as SCN. Faske reported that fluopyram reduced galling in tomatoes resulting from *M. incognita* (2014).

The main objective of this study is to evaluate fluopyram and its ability to suppress both SCN and SDS separately and together. To address these objectives, three sets of experiments were completed: examine the effects of ILeVO® on SCN alone, SDS alone and both pathogens simultaneously.

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CHAPTER 2. EFFECTS OF ILeVO® ON SOYBEAN CYST NEMATODE

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Abstract

Heterodera glycines or the soybean cyst nematode (SCN) is a pathogen of major concern to soybean farmers especially in the Midwest. Until recently, SCN management was limited to host resistance and cultural practices. In recent years, seed treatments have been marketed for the management of several pathogens including SCN. An experimental seed treatment called ILeVO® is set to come to market in the spring of 2015. The purpose of this study was to test the effectiveness of ILeVO® on SCN. To do so, plants were grown in soil naturally infested with SCN. Seeds were treated with a variety of seed treatment combinations with and without ILeVO®. Plants were grown in water baths in a greenhouse in three runs of the experiment. After 30 days, SCN females were collected from roots and counted. A combination of Trilex®+ Allegiance®+ Poncho/VOTiVO®+ ILeVO® had fewer SCN females per gram of wet root mass than the combination of Trilex®+ Allegiance®+ Poncho/VOTiVO®. There was also a negative effect of ILeVO® on root mass in the presence of SCN. These data suggest that ILeVO® may be effective against SCN.

Introduction

Soybean cyst nematode continues to be the most harmful pathogen on soybeans in the United States (Koenning and Wrather, 2010; Wrather and Koenning, 2006). Between 2006 and 2009, SCN accounted for an estimated 34 million metric ton reduction in soybean yield in the

United Sates (Koenning and Wrather, 2010). Due to the persistent yield losses over time, it is imperative that new management strategies are developed for the continued, successful management of this pathogen and reduction of its impact in years to come.

The first report of SCN was in Japan in 1915 (Hori, 1916). Supposedly introduced on infected bulbs from Japan, SCN was discovered in the United States in 1954 (Winstead et al., 1955). SCN rapidly spread and was soon distributed throughout much of the United States (Riggs, 1977). The modes of transmission for SCN include but are not limited to: movement of infested soil on farm equipment, drainage, wind and birds (Wrather et al., 1984; Epps, 1971). Due to the ease of movement from field to field, this pathogen is very widespread (Tylka and Marett, 2014) and will continue to persist as long as host crops are grown.

Aside from the easy dissemination of SCN, the short duration of the pathogen's life cycle allows for rapid population growth. The SCN life cycle begins with eggs in a dead female, or cyst. This cyst encapsulates the eggs over winter in the absence of host plants. Once the seasonal diapause of the eggs has ended, hatching begins when soil temperatures reach approximately 24°C (Ross, 1964). Upon hatching, juveniles are drawn towards root exudates and then penetrate the root to feed and reproduce. On average, each SCN female produces 100 to 200 eggs (Schmitt et al., 2004). After the SCN life cycle is complete, females will form into cysts and detach from the host.

SCN populations can complete several generations over one growing season (Wrather et al., 1984). The number of life cycles during a season depends on the soil environment. According to Wrather et al. (1984), SCN can complete its life cycle in 24 days at an average soil temperature of 23˚C and 40 days at 18˚C. SCN population density is also affected by soil environment. A study by da Rocha et al. (2008) found that 26˚C yielded the highest numbers of

SCN females found on a plant across a range of temperatures between 20 and 35˚C. If conditions are optimal, SCN can generate very large population densities in a field that will be an issue in subsequent years when soybeans are grown.

The damage caused by SCN is variable based on population densities present at the beginning of a growing season. Francl and Dropkin (1986) found that yield loss was observed with an egg count of 470 eggs per kilogram of soil at the beginning of the season. More recent assessments made by Iowa State University extension suggests that in soil where soybean will be the next crop; low infestation is 1-2000 eggs per 100 cm^3 of soil, 2,001-12,000 for moderate infestation and >12,000 for high infestation ("Soybean cyst nematode management field guide", 2008). To reduce the yield loss exhibited by SCN, scouting and management are required.

For many years, management of nematodes in highly valued crops was accomplished by injecting chemicals, such as methyl bromide, into the soil (Martin, 2003; Noling and Becker, 1994). The use of these pesticides was slowly discontinued due to the environmental implications of their use. For SCN management in large-scale agriculture, this left cultural practices such as rotation to a non-host crop and the use of resistant cultivars (Howard et al., 1998; Niblack, 2005; Schmitt, 1991; Wrather et al.1984). Growing non-host crops creates a longer period in which SCN eggs hatch and juvenile nematodes do not have a host to infect, reducing overall population density through starvation.

The use of a resistant cultivar is one form of management that can further reduce nematode population densities. A resistant cultivar allows <10% reproduction when compared to a standard susceptible soybean (Schmitt and Shannon, 1992). Currently, most of SCN-resistant cultivars are derived from a single source of resistance (PI 88788) (Tylka and Mullaney, 2013).

With this limitation to the diversity of host resistance, other management strategies may become important.

Ideally, the most effective management of any pathogen would be an integration of several tactics to minimize yield loss and slow the process of the pathogen adapting to render any one of these practices less effective. At times, rotating with a non-host and using resistant cultivars may not be enough to reduce yield losses. Using chemical or biological seed treatments that reduce SCN infection may be a possible management tool to complement crop rotation and resistant cultivars.

In recent years, the use of seed-applied nematicides and nematode repellents has become widespread (Faske and Starr, 2007; Frye, 2009; Monfort et al., 2006). Due to the nature of SCN infecting early in the plant life cycle, seed treatments could be a successful form of chemical delivery to manage soil-borne nematodes, such as SCN. According to Lawn and Noel (1986), SCN population densities are at their greatest six weeks after planting and then decline suggesting that seed treatments could be effective. Seed treatments that are taken up systemically and persist on the root surface should be most useful with regards to SCN management.

The goal of this research is to look at the effects of the seed treatment ILeVO® on SCN. Anecdotal evidence has shown that the succinate dehydrogenase inhibitor (SDHI), fluopyram (the active ingredient in ILeVO®), may have nematicidal effects.

Materials and Methods

Experimental design

This experiment was completed three times under greenhouse settings within temperature-controlled water baths. For each run, the experimental design was a two-way,

complete factorial treatment arrangement. Six replicates of each of the 28 treatment-cultivar combinations were used. The 28 treatments consisted of four soybean cultivars each treated with seven different combinations of seed treatments. Four soybean cultivars were selected based on their level of susceptibility to the SCN population in the soil collected from Oskaloosa, Iowa (Table 2.1). The four levels of susceptibility were based on comparisons to a known susceptible cultivar (Lee 74) (Schmitt and Shannon, 1992). Each of the four cultivars was treated with seven different seed treatment combinations, provided by Bayer CropScience (Research Triangle, NC) (Tables 2.2 and 2.3). The seed treatment combinations selected were those that were being considered for market on soybean by Bayer CropScience at the time of the study.

Three runs of the experiment were performed in August 2012, December 2012 and May 2013. An initial egg count was determined for the soil by processing four 100-cubic centimeters (cm^3) samples of soil (Faghihi and Ferris, 2000; Gerdemann, 1955) and averaging the egg counts obtained. Due to low initial numbers of eggs (<1,000 eggs/100cm^3 of soil), SCN population densities were increased by growing susceptible Pioneer 93M11 soybeans in the soil for 30 days at ambient greenhouse temperature to allow for one generation of SCN to be produced. After 30 days, plant tops were cut off the plants and discarded while root masses were allowed to dry within the soil. Soil with the newly cultured SCN was then incorporated into the original Oskaloosa soil and eggs were counted and averaged again. Soil was mixed with sand to achieve the final number of SCN eggs per 100cm^3. The initial SCN egg population was 8,313 eggs/100cm^3 of soil for the first run and 4,500 eggs/100cm^3 of soil for the second and third runs of the experiment.

The SCN-infested soil was used to fill the cone-tainers that were 1.27 cm in diameter, 21 cm in depth and held a volume of 164 cubic centimeters (Stuewe & Sons, Inc., Tangent, OR).

Seeds of each treatment were planted approximately 3.5 cm deep, and then covered with sand to the top of the cone-tainer. Cone-tainers were labeled, and then placed into randomly determined positions in 5 gallon plastic storage containers, which were filled with sand to distribute temperature for all of the cone-tainers.

The water baths were maintained at 27˚C (80.6˚F). This temperature was used based on previous work showing that the optimal temperature for embryogenesis and hatching of SCN was between 24 and 30˚C (Alston and Schmitt, 1988). Soil temperature was measured in two randomly selected cone-tainers in one experimental run. The average temperature was 27.0˚C with ranges of 25.3-29.5˚C. Six 5 gallon plastic storage containers were placed into the water bath for each run.

Plants were watered as needed, with an effort to avoid excess moisture. If the seed coat emerged attached to the cotyledon once seeds germinated, it was placed into the corresponding cone-tainer on the soil surface. This was to ensure that any product left on the seed coat remained in the correct cone-tainer. After 30 days, plants were removed from cone-tainers and processed.

Data collection

To collect SCN data, the roots were gently rinsed to remove a majority of the soil particles. Roots were then sprayed over a pair of sieves (20-mesh with 841µm pores nested above a 60-mesh with 250µm pores) to collect SCN females, which were then counted (Gerdemann, 1955). Each sample was further processed to determine the number of eggs per sample (Faghihi and Ferris, 2000). Females were washed onto a 250 µm-pore sieve and ruptured by grinding with a rubber stopper. The ground SCN female suspension was rinsed through a 200-

mesh (74µm pores) sieve nested over 500-mesh (25µm pores) sieve. Eggs were stained (Bybd et al., 1983) and counted using a standard dissecting microscope.

Dry and wet root mass were recorded for each plant so that they could later be used to quantify the number of SCN females and eggs per gram of root mass. Each root sample was blotted dry and weighed to obtain the wet root mass, then root samples were placed into paper sacks and dried in an oven at 90˚C for two days and weighed to determine dry root mass.

Statistical analyses

All data in these three experiments were analyzed using SAS (Version 9.2, SAS Institute Inc., Cary, NC). PROC GLIMMIX was used to analyze the data, with the interaction of experimental run and replicate set as a random effect. Cultivar and seed treatment were fixed effects. Data collected for the number of SCN females and the number of eggs per sample were log transformed (natural log) to normalize the data. A Tukey-Kramer adjustment was used to account for experiment-wise error. Finally, contrasts were made between Trilex®+Allegiance®+ILeVO® and Trilex®+Allegiance®, as well as between Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO® and Trilex®+Allegiance®+Poncho/VOTiVO® to test the addition of ILeVO® to these combinations.

Results

The cultivar main effect was significant for all of the response variables (Table 2.4). As expected, Pioneer 93Y13 (SCN resistant) had the lowest number of SCN females and SCN eggs both per gram of root and per plant, compared to the other three cultivars (Table 2.5). SCN populations increased as susceptibility increased for the other three cultivars. With regards to wet

root mass, the Pioneer 93M11 plants had significantly smaller roots (P<0.001) followed by the Pioneer 93Y13, Asgrow 3432 and Asgrow 3231.

No cultivar by seed treatment interaction was observed for any response variables (P>0.05) (Table 2.4). There was a highly significant difference for the run-cultivar interaction for wet root mass (P<0.001), suggesting that root mass reaction be separated by cultivar for each run (data not shown). However, there was not a significant interaction between run and seed treatment, so the data were combined for the analysis of the seed treatments. The main effect for seed treatment was highly significant (P<0.001) for wet root mass (Table 2.4). The seed treated with the Trilex® + Allegiance® + Poncho/VOTiVO® + ILeVO® had 22% less wet root mass compared to seed treated with only Trilex® + Allegiance®.

When the numbers of SCN females and eggs were compared on a per gram of wet root mass basis, the main effects for seed treatment were significant for females per gram of root mass (P=0.023) (Table. 2.4). However, after the Tukey-Kramer adjustment this difference was no longer present. The main effect of seed treatment was significant, for the number of eggs (P=0.043) and SCN females (P=0.006) per plant (Table 2.4). There was a 35% reduction in SCN females per plant for Trilex® + Allegiance® + Poncho/VOTiVO® + ILeVO® compared to red colorant (Table 2.6).

Specific pairwise comparisons were examined between Trilex®+Allegiance® and Trilex® + Allegiance® + ILeVO® as well as between Trilex® + Allegiance® + Poncho/VOTiVO® and the Trilex® + Allegiance® + Poncho/VOTiVO® + ILeVO® treatments (Table 2.7). These comparisons were made to determine the potential effect of the ILeVO®. Root mass from plants treated with combinations including ILeVO® were significantly smaller for both comparisons (Table 2.6). The Trilex® + Allegiance® + Poncho/VOTiVO® treatments showed a 27% increase in numbers

of SCN females per plant and a 22% increase in eggs per plant when compared to the Trilex® + Allegiance® + Poncho/VOTiVO® + ILeVO® combination. For the comparisons on a per gram of wet root mass basis, the Trilex® + Allegiance® + Poncho/VOTiVO® + ILeVO® reduced the number of SCN females by 20% when compared to Trilex® + Allegiance® + Poncho/VOTiVO® (Table 2.6).

Discussion

Based on these results, there is evidence that ILeVO® may have some effect on SCN. When all of the seed treatment combinations in this study were compared as a whole, no significant effect of seed treatments on SCN population densities was detected. However, the pairwise comparisons made between Trilex® + Allegiance® + Poncho/VOTiVO®, with and without ILeVO®, revealed a 20% reduction in the number of SCN females per gram root mass in the presence of ILeVO®. The same reduction was not seen, however, for the comparison of Trilex® + Allegiance® with and without ILeVO®.

One limitation of this experiment was the pre-established treatment combinations that were available for use in the study. Ideally, seed treated with a single product would have been better to use and the results simpler to interpret, since the other products in the experimental treatments add uncertainty. However, commercially available seed is sold with a pre-established combination of chemicals each with an intended target, such as insects, fungi, and weeds. Trifloxystrobin (Trilex®) has been well studied for the treatment of many fungal diseases, including powdery mildew (Reuveni, 2000; Reuveni, 2001), sugar beet diseases (Watanabe et al., 2006) and even as a protective measure against certain abiotic stresses (Han, 2012). Trifloxystrobin has not been shown to affect nematodes. Metalaxyl (Allegiance®) has been

commonly used to prevent *Phytophthora* (Ioannou and Grogan, 1984), *Pythium* (Hwang et al., 1996) and nematodes on citrus (Kaplan, 1983). As shown by Schrimsher et al. (2011), *Bacillus firmus* (VOTiVO®) affects juvenile SCN. Clothianidin (Poncho®) is a neonicotinoid seed-treatment insecticide intended to control insects and has been shown to work synergistically with certain entomopathogenic nematodes (Koppenhofer and Fuzy, 2008).The combinations in this experiment are designed to give a broad range of protection. In combination, it is difficult to determine if one chemical or biological agent is acting with more influence than others or if the products are interacting. However, the reason we used these seed treatment combinations was that ILeVO® will likely to be marketed in these combinations and so these combinations were what Bayer CropScience provided for testing. Further testing of ILeVO® would be necessary to draw further conclusions.

Another possible shortcoming of this experiment is that it was conducted in a greenhouse whereas field conditions have a much higher level of variability. This greenhouse experiment conveys the SCN life cycle under optimal conditions. However, the number of SCN used to inoculate plants was within the normal ranges of moderate SCN infestation ("Soybean cyst nematode management field guide", 2008). This shows that the level of inoculum used in the experiment is attainable in the field. Had we used a high level of inoculum, SCN population growth may have exceeded what could be seen under field conditions since the temperature was held at an optimum. The greenhouse settings we used for this experiment do not seem to be beyond reasonable possible field scenarios.

An additional point of interest is that there were no significant differences found for the interaction of cultivar and seed treatment for any response variables ($P > 0.05$) (Table 2.4). This finding demonstrates that the differences observed were the same across all four cultivars. Also,

three of the four cultivars are derived from the PI 88788 source of resistance for SCN, (Pioneer 93M11 did not have any SCN resistance, it was susceptible) and they were all in a similar maturity group (Tylka and Mullaney, 2013; Tylka et al., 2014). Since the vast majority of SCN-resistant cultivars have the same source of resistance (PI 88788), ILeVO® may have an effect on SCN when paired with most SCN-resistant cultivars. Further testing of cultivars with different sources of SCN resistance should be done to make further conclusions.

ILeVO® also may negatively affect early season soybean root mass. As a whole, the seed treatment combination containing all of the chemicals had the lowest root mass. Also, when comparisons were made for the combinations with and without ILeVO®, the presence of ILeVO® had a negative effect on root weight in the comparison of Trilex® + Allegiance® + Poncho/VOTiVO® versus Trilex® + Allegiance® + Poncho + VOTiVO® + ILeVO®. These data suggest that there is a negative correlation between ILeVO® and plant root mass. Reduction of root mass can lead to a decrease in a plants ability to take in water. A study by Fiscus and Markhart (1979) showed that root size played a major role in the amount of water a plant was able to take in. Diminished root size, coupled with the risk of moisture stress, could be a yield-limiting factor. According to Boyer (1982), drought accounted for more crop losses than any other abiotic factor between 1939 and 1978. However, the decreased root mass may be influenced by the amount of available space for roots to grow. Each cone-tainer holds 164cm^3 of soil and may have limited the amount of root growth for each plant. This confined area may also have influenced the amount of active ingredients that were in the direct proximity of the roots.

Prompted by this finding, a separate study was performed in the greenhouse looking at the effects of each seed treatment combination on root mass. The findings of this study were that

the effect of seed treatment was significant, however, contrasts show that the presence of ILeVO® did not have a negative correlation with root mass (Appendix A).

Conclusions

In conclusion, ILeVO® may have an effect on SCN, but may also contribute to root mass reductions in plants. How these factors affect yield and plant development is unknown. In future experiments, it would be helpful to find out how the SDHI, ILeVO®, is affecting this nematode. It would also be helpful to be able to test ILeVO® alone without the other products in combination. Singling out the product would give a better insight into whether or not ILeVO® is affecting SCN activity. It would also be helpful to test different populations of SCN and different temperatures. Finally, it would be useful to test the product on soybean cultivars with different sources of resistance.

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Tables and Figures

Table 2.1 Cultivar names and susceptibility to soybean cyst nematode (SCN) collected in Oskaloosa, Iowa

Cultivar	SCN reaction [z]
Pioneer 93Y13	resistant (<10% reproduction)
Asgrow AG 3231	moderately resistant (10-30%)
Asgrow AG 3432	moderately susceptible (31-60%)
Pioneer 93M11	susceptible (>60%)

[z] SCN reaction is determined as the percentage of reproduction when compared to SCN reproduction on Lee 74

Table 2.2 Seed treatment products from Bayer CropScience and their effectiveness towards soybean cyst nematode (SCN)

Trade name	Active Ingredient	SCN activity	Rate of seed treatment	FRAC code [z]
Trilex®	trifloxystrobin	no	5g active/ 100kg of seed	11
Allegiance®	metalaxyl	no	4g active/ 100kg of seed	4
Poncho®	clothianidin	no	125g active/ 100kg of seed	
VOTiVO®	*Bacillus firmus*	yes	5 million units/ seed	
Poncho/VOTiVO®	clothianidin/ *Bacillus firmus*	yes	0.13mg active/ seed	
ILeVO®	fluopyram	unknown	0.15mg active/ seed	7

[z] Fungicide resistance action committee=FRAC

Table 2.3 Treatment list, used to evaluate SDS and SCN infection in the presence of ILeVO®

Treatment number	Product combination [z]
1	red colorant (untreated control)
2	Trilex®+Allegiance®
3	Trilex®+Allegiance®+VOTiVO®
4	Trilex®+Allegiance®+Poncho®
5	Trilex®+Allegiance®+Poncho/VOTiVO®
6	Trilex®+Allegiance®+ILeVO®
7	Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO®

[z] All seed treatment combinations include red colorant

Table 2.4 ANOVA summary table for data across all three runs of the experiment evaluating the effects of ILeVO® on soybean cyst nematode (SCN)

Source	Degrees of freedom	Sum of squares	Mean square	F value	P value
Wet root mass (g)					
Replicate	5	2.64022	0.528	1.410	0.306
Run	2	48.043	24.022	64.020	<.0001
Seed treatment	6	6.616	1.103	5.040	<.0001
Cultivar	3	19.616	6.539	29.860	<.0001
Seed treatment x cultivar	18	2.024	0.112	0.510	0.952
Seed treatment x run	12	1.476	0.123	0.560	0.872
Cultivar x run	6	6.426	1.071	4.890	<.0001
Seed treatment x cultivar x run	36	6.428	0.179	0.820	0.768
Rep(run)	9	3.375	0.375	1.710	0.085
Residuals	336	73.571	0.219		
SCN females per plant [z]					
Replicate	5	5.433	1.087	1.250	0.363
Run	2	31.955	15.977	18.160	0.001
Seed treatment	6	6.109	1.018	3.120	0.006
Cultivar	3	215.169	71.723	219.910	<.0001
Seed treatment x cultivar	18	3.648	0.203	0.620	0.883
Seed treatment x run	12	5.512	0.459	1.410	0.160
Cultivar x run	6	3.894	0.649	1.990	0.067
Seed treatment x cultivar x run	36	5.166	0.144	0.440	0.998
Rep(run)	9	7.902	0.878	2.690	0.005
Residuals	332	108.279	0.326		

Table 2.4 continued

SCN females per gram of root [zy]					
Replicate	5	6.800	1.360	0.920	0.509
Run	2	83.949	41.975	28.120	0.000
Seed treatment	6	5.304	0.884	2.490	0.023
Cultivar	3	281.004	93.668	263.350	<.0001
Seed treatment x cultivar	18	3.906	0.217	0.610	0.892
Seed treatment x run	12	6.507	0.542	1.520	0.113
Cultivar x run	6	0.807	0.134	0.380	0.893
Seed treatment x cultivar x run	36	6.966	0.194	0.540	0.986
Rep(run)	9	13.405	1.489	4.190	<.0001
Residuals	331	117.730	0.356		
SCN eggs per plant [z]					
Replicate	5	3.502	0.700	0.590	0.712
Run	2	7.270	3.635	3.010	0.100
Seed treatment	6	4.556	0.759	2.200	0.043
Cultivar	3	457.337	152.446	441.160	<.0001
Seed treatment x cultivar	18	6.174	0.343	0.990	0.468
Seed treatment x run	12	7.418	0.618	1.790	0.049
Cultivar x run	6	4.046	0.674	1.950	0.072
Seed treatment x cultivar x run	36	7.458	0.207	0.600	0.968
Rep(run)	9	10.843	1.205	3.490	0.000
Residuals	336	116.108	0.346		
SCN eggs per gram of root [zy]					
Replicate	5	5.336	1.067	0.710	0.630
Run	2	48.527	24.264	16.060	0.001
Seed treatment	6	3.289	0.548	1.480	0.183
Cultivar	3	564.199	188.066	508.550	<.0001
Seed treatment x cultivar	18	6.866	0.381	1.030	0.423
Seed treatment x run	12	8.178	0.681	1.840	0.041
Cultivar x run	6	1.385	0.231	0.620	0.711
Seed treatment x cultivar x run	36	8.153	0.226	0.610	0.963
Rep(run)	9	13.582	1.509	4.080	<.0001
Residuals	335	123.887	0.370		

[z] values were log transformed for statistical analyses

[y] values were calculated based on wet root mass

Table 2.5 Tukey-Kramer estimates for main effect of all cultivars on mean response variables, for all three runs of the experiment evaluating the effects of ILeVO® on soybean cyst nematode (SCN)

Cultivar	Wet root mass (g) [y]	SCN females per plant [zy]	SCN eggs per plant [zy]	SCN females per gram of root [zyx]	SCN eggs per gram of root [zyx]
Pioneer 93Y13	1.62 b	22 c	2,331 c	15 d	1,527 d
Asgrow AG 3231	1.89 a	44 b	4,196 b	25 c	2,399 c
Asgrow AG 3432	1.70 b	50 b	5,032 b	32 b	3,203 b
Pioneer 93M11	1.29 c	167 a	37,983 a	137 a	31,549 a

[z] values were log transformed for statistical analyses and back transformed to present in tables
[y] Estimates with different letters are statistically different ($P<0.05$)
[x] values were calculated based on fresh root mass

Table 2.6 Tukey-Kramer estimates for main effect of all seed treatment combinations on mean response variables, for all three runs of the experiment evaluating the effects of ILeVO® on soybean cyst nematode (SCN)

Seed treatment	Wet root mass (g) [y]	SCN females per plant [zy]	SCN eggs per plant [zy]	SCN females per gram of root [zyx]	SCN eggs per gram of root [zyx]
Red colorant	1.60 abc	63 a	6,836 ab	42 a	4,582 a
Trilex®+Allegiance®	1.80 a	51 ab	6,905 ab	31 a	4,105 a
Trilex®+Allegiance®+VOTiVO®	1.76 ab	52 ab	6,248 ab	32 a	3,828 a
Trilex®+Allegiance®+Poncho®	1.62 abc	56 ab	6,905 ab	37 a	4,629 a
Trilex®+Allegiance®+Poncho/VOTiVO®	1.53 bc	56 a	6,768 ab	40 a	4,817 a
Trilex®+Allegiance®+ILeVO®	1.66 abc	57 a	7,332 a	37 a	4,769 a
Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO®	1.41 c	41 b	5,271 b	32 a	4,024 a

[z] values were log transformed for statistical analyses and back transformed to present in tables
[y] estimates with different letters are statistically different ($P<0.05$)
[x] values were calculated based on fresh root mass

Table 2.7 P-values for pairwise contrasts between seed treatment combinations with and without ILeVO® across all response variables for the combined three runs of the experiment evaluating the effects of ILeVO® on soybean cyst nematode (SCN)

Contrast	Wet root mass (g)	SCN females per plant [z]	SCN eggs per plant [z]	SCN females per gram of root [zy]	SCN eggs per gram of root [zy]
Trilex®+Allegiance®+Poncho/VOTiVO® vs. Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO®	0.017	0.003	0.016	0.031	0.097
Trilex®+Allegiance® vs. Trilex®+Allegiance®+ILeVO®	0.113	0.307	0.523	0.079	0.168

[z] values were log transformed for statistical analyses
[y] values were calculated based on fresh root mass

CHAPTER 3. EFFECTS OF ILeVO® ON SOYBEAN SUDDEN DEATH SYNDROME AND SOYBEAN CYST NEMATODE

A paper to be submitted to *Plant Disease*

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Abstract

Soybean sudden death syndrome (SDS) is a common and widespread soybean disease across the soybean-growing states in the United States Currently there are no chemical control methods for SDS, limiting management to host resistance and cultural practices. In recent years, a fungicide seed treatment with the active ingredient fluopyram (registered as ILeVO®) has been evaluated for possible management of SDS. The purpose of this study is to test the effects of ILeVO® on SDS and the combination of SDS and soybean cyst nematode (SCN). To study these effects, plants were grown in water bath maintained at 24˚C in a greenhouse. Seed was treated with one of seven seed treatment combinations. This experiment was repeated three times. Plants were divided into two groups: those to be inoculated with *Fusarium virguliforme* or plants to be inoculated with *F. virguliforme* and SCN. These two groups of plants were then inoculated with the appropriate pathogens and grown for 35 days. Our analysis did not find a significant reduction in the amount of foliar SDS severity in the presence of ILeVO®, regardless of the presence of SCN. ILeVO® did show a reduction in root rot severity for the contrast of Trilex® + Allegiance® + ILeVO® and Trilex® + Allegiance®. Also, there was a reduction of SCN females per gram of wet root mass for the contrasts of Trilex® + Allegiance® + Poncho/VOTiVO® + ILeVO® vs. Trilex® + Allegiance® + Poncho/VOTiVO® and Trilex® + Allegiance® + ILeVO® vs. Trilex® + Allegiance® for run number one. There was also a reduction in SCN females per gram

of wet root mass for the contrast of Trilex® + Allegiance® + ILeVO® and Trilex® + Allegiance®. These results suggest there is not an effect of ILeVO® on SDS foliar symptoms, but there may be an effect on SCN.

Introduction

Soybean sudden death syndrome (SDS) is a prominent and devastating disease on soybeans. In 2010, a particularly bad year for SDS, an estimated 4.7 million metric tons yield loss occurred across the United States (Bradley and Koenning, 2014). There are few management options for SDS, and a more diverse selection is needed to help limit losses from this disease.

SDS was first observed, in the United States, in 1971 by H.J. Walters who noticed plants with chlorotic lesions in a field in Arkansas (Roy et al. 1997). The disease was first named sudden death syndrome in 1982 by Hirrel (1983). Four different species of *Fusarium* have been identified that cause SDS; *F. virguliforme* is the only causal agent found in the United States (Aoki et al., 2005). Currently SDS can be found in Arkansas, Illinois, Indiana, Iowa, Kansas, Kentucky, Michigan, Minnesota, Mississippi, Missouri, Nebraska, Ontario, South Dakota, Tennessee, and Wisconsin (Anderson and Tenuta, 1998; Bernstein et al., 2007; Chilvers and Brown-Rytlewski, 2010; Jardine and Rupe, 1993; Kurle et al., 2003; Tande et al., 2014; Roy et al., 1989; Rupe, 1989; Yang and Rizvi, 1994; Ziems et al., 2006).

The causal agent of SDS is a soil-borne pathogen that can be spread through contaminated farm equipment (Yang and Lundeen, 1997). McLean and Lawrence (1993) also found that *F. virguliforme* could colonize SCN cysts and eggs. The SDS disease cycle begins with the infection of soybean roots by germinating chlamydospores, which are the overwintering

structure that can persist in a wide range of temperatures and soil conditions (Westphal et al., 2008). In the spring, chlamydospores develop hyphae, which are able to infect soybean plants (McLean and Lawrence, 1995). Studies have shown that plants infected at the time of planting develop the worst foliar symptoms, while older plants are less susceptible to SDS root infection (Gongora-Canul and Leandro, 2011; Gongora-Canul and Leandro, 2011). After infection, symptoms develop as discolored roots and when soil is wet, blue spore masses can sometimes be observed on the taproot, when removed from the soil.

The other SDS symptom is on the leaves, and in many cases appears long after the plant roots have been infected, towards the latter half of the growing season (Luo et al., 1999; Roy et al., 1997). Foliar symptoms are interveinal chlorotic lesions, which may eventually become necrotic. Foliar symptoms are caused by *F. virguliforme* producing a toxin in the roots (Brar et al., 2011; Jin et al., 1996; Jin et al., 1996) and moving the toxin through the vascular system to the foliage.

There are certain environmental conditions that favor *F. virguliforme* infection and SDS development. The optimal temperature for root infection is 15-17˚C (Gongora-Canul and Leandro, 2011; Scherm and Yang, 1996). This temperature range, however, is not the same for the development of foliar symptoms. Optimal temperatures for SDS foliar symptom development is around 22-25˚C (Gongora-Canul and Leandro, 2011; Scherm and Yang, 1996). The difference in optimal temperatures for each phase of the disease explains why SDS root rot typically occurs early in the season and foliar symptoms occur later in the season.

Currently there are no fungicide seed treatments on the market for managing SDS (Weems et al., 2011). All management, thus far, has been through cultural practices and host resistance. According to Leandro et al. (2013), SDS can develop on any cultivar under optimal

environmental conditions. Other management strategies include tillage, delayed planting, and rotation to non-host crops. Delaying planting until later in the growing season reduces SDS severity (De Bruin and Pederson, 2008; Hershman et al., 1990; Wrather et al., 1995), but may reduce yield potential since the duration of the growing season is shortened (De Bruin and Pederson, 2008). Short-term crop rotation, such as the typical corn-soy rotation, has proven ineffective for the reduction of SDS (Westphal and Xing, 2011; Xing and Westphal, 2009). According to Abdelsamad et al. (2012), long-term rotations including other crops can reduce SDS.

Not only does SDS cause significant damage on its own, there is also evidence that SDS interacts with *Heterodera glycines,* the soybean cyst nematode (SCN) (Gao et al., 2006; McLean and Lawrence, 1995; Roy, 1989; Xing and Westphal, 2006, Xing and Westphal, 2009). The presence of SCN in a field increases the severity of SDS (McLean and Lawrence, 1995; Roy, 1989).

The goal of this experiment is to study the effects of ILeVO® on SDS alone and in the presence of SCN. It has been reported that there is an effect of ILeVO® on SDS in the field (Mueller et al., 2011). This study will specifically address the effects of ILeVO® on SDS under greenhouse conditions, alone and with a controlled amount of SCN.

Materials and Methods

Experimental design

This set of experiments examined seven seed treatments on soybean that were inoculated with *F. virguliforme* or both *F. virguliforme* and SCN. The experimental design was a split plot, dividing two sets of cone-tainers (Stuewe & Sons, Inc., Tangent, OR), using the two-way complete factorial treatment arrangement. A preliminary study determined that SCN could move

from cone-tainers with SCN to cone-tainers without SCN, in a similar experimental setup (data not shown). So each 5 gallon plastic storage container was split into two sections using a plastic bag to divide cone-tainers with *F. virguliforme* alone and in combination with SCN. Dividing the two sets of pathogens ensured that SCN did not transfer to cone-tainers intended for SDS alone. The experiment was completed three times, each with six replications.

Plants were grown individually in cone-tainers that were 3.8 cm in diameter, 21 cm in depth and held a volume of 164 cubic centimeters. Cone-tainers were randomized into a 5 gallon plastic storage container filled with sand to maintain the same temperature for all cone-tainers. All runs of the experiment were performed in a greenhouse within temperature-controlled water baths set to 24˚C (74˚F) to facilitate both the development of SDS and infection and reproduction of SCN (Scherm and Yang, 1996; Gongora-Canul and Leandro, 2011).

Inoculum preparation

The source of inoculum for all experiments was *F. virguliforme* "Mont1". Inoculum was prepared in a sorghum base, adapted from a procedure created by Hartman et al. (1997), which was originally designed for use with red sorghum and mycelial plugs of *Fusarium*. Our protocol used 2,280 cm^3 of white sorghum seed with 10 ml of water added. This mixture was then autoclaved twice for one hour at 121˚C. After the mixture was cooled, 2 ml of a 10^6 spores/ml spore suspension were added. *F. virguliforme* was incubated at room temperature (estimated at 22˚C) for one week, briefly mixing every other day by massaging the bag. Inoculum was then plated on potato dextrose agar (PDA), which was amended with streptomycin and tetrachlorocycline to prevent bacterial growth, to check that there was no fungal growth other than *Fusarium virguliforme*. A preliminary experiment was performed testing different

substrates for the growth of *F. virguliforme* and the ability of SCN to infect the plant when the substrate was present (Appendix B). Whole sorghum seed that was mixed into pasteurized soil similar to the procedure carried out by Luckew et al. (2012) at a rate of 1:30 sorghum: pasteurized soil produced the best SDS foliar symptom severity and still allowed adequate SCN infection.

The ratio from our preliminary study was increased to 1:20 to ensure SDS foliar symptom severity was high. Also, soil from Oskaloosa, Iowa with SCN was used to naturally infest half of the experiment. For the portion of the experiment infested with only *F. virguliforme,* Oskaloosa soil was pasteurized to eliminate SCN from the soil. This soil was tested for SCN by planting the susceptible cultivar Pioneer 93M11, and no SCN was observed on the roots (data not shown).

Adjustments were made each time the experiment was completed to balance between *F. virguliforme* infection and SCN reproduction. For the first run, *F. virguliforme* inoculum was produced using whole sorghum, which was not soaked 24 hours prior to autoclaving. Subsequent experimental runs used white sorghum inoculum that was pre-soaked in water prior to autoclaving. Soaking the sorghum prior to autoclaving, we think, resulted in a more suitable environment for culturing the *Fusarium.* Plants were inoculated with a 1:20 ratio of inoculum: soil for the first two runs and the third run was inoculated at a rate of 1:30.

Population densities of SCN for the Oskaloosa field soil were 1,000 eggs/100cm^3 of soil. Populations were increased by growing the susceptible Pioneer 93M11 soybeans in clay pots for 30 days. Plant tops were then clipped off and the soil left to dry. The soil was then mixed into the original Oskaloosa soil until a moderate SCN population density was reached. The first run of the experiment had an initial eggs count of 6,175 eggs/100cm^3 of soil, and runs two and three were 4,200 eggs/100cm^3 of soil.

Experimental procedure

Soil was partitioned into twelve aliquots, six naturally infested with SCN and six pasteurized, and all twelve aliquots were mixed with sorghum infested with *F. virguliforme*. Two soybean cultivars were used in this experiment – Pioneer 93Y13 and Pioneer 93M11 (Table 3.1). A preliminary greenhouse experiment revealed that these two cultivars did not differ in their susceptibility to SDS (Appendix C). Eight seed treatment-cultivar combinations were used for this experiment including an untreated seed as a control (Tables 3.2 and 3.3). The treatment combinations used were those that were being marketed by Bayer CropScience at the time of the study.

Plants were allowed to grow for 35 days. This duration was chosen to give SCN enough time to complete a single generation. A preliminary experiment was performed looking at the time it took SCN to complete a generation at the lower, less optimal temperature of 24˚C to ensure that enough SCN reproduction was occurring at that point (Appendix D). Plants received 10 ml of water every day to keep the amount of soil moisture consistent between cone-tainers.

Data Collection

During the 35 day incubation period, SDS foliar symptoms severity was measured every other day upon the appearance of the first foliar symptoms, as a percentage of leaf area that was chlorotic or necrotic. In the instance that a leaf fell off a plant, the remaining leaves were rated based on the percentage of the leaf area infected. At the end of each experimental run, the area under disease progress curve was calculated as a measure of foliar symptom severity over

time $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti + 1 - Ti)$, where Y_i= the initial amount of disease and T_i=the initial time disease was recorded (Simko and Piepho, 2012).

Following each run, plant roots were rinsed free of soil and scanned using an Epson Expression 10000XL scanner and the WinRHIZO software package (Regent Instruments Canada Inc.) and visually rated for the prevalence of root rot. Scanning was done to examine the number of root tips per plant. After being scanned, roots were sprayed through a 20-mesh sieve with 841µm pores nested above a 60-mesh sieve with 250µm pores to remove SCN females (Gerdemann, 1955). Females were then ground open to release eggs into a 200-mesh (74µm pores) sieve nested over 500-mesh (25µm pores) sieve (Faghihi and Ferris, 2000), and eggs were then stained (Bybd et al., 1983) and counted.

After the roots were sprayed free of SCN females, wet root mass was recorded. Roots were then placed into a drying oven at 90˚C for two days then weighed again. The root mass was used to determine the number of SCN females and eggs per gram of root mass.

Statistical Analyses

The experimental design was a split plot, using the three-way complete factorial treatment arrangement including pathogen set, cultivar and seed treatment. The whole-plot for the experiment was the division of the two pathogen sets and the split-plot contained the cultivar by seed treatment combinations. Data were analyzed using SAS (Version 9.2, SAS Institute Inc., Cary, NC). PROC GLIMMIX was used to allow for the ability to treat the interaction of pathogen set and replicate as a random effect. The Tukey-Kramer adjustment was used to account for experiment-wise error. To normalize certain response variables, the natural log of the data was taken for the analysis, and then back transformed means were presented in tables.

Finally, contrasts were made between Trilex® + Allegiance® + ILeVO® versus Trilex® + Allegiance® as well as Trilex® + Allegiance® + Poncho/VOTiVO®+ ILeVO® versus Trilex® + Allegiance® + Poncho/VOTiVO® to isolate the possible effects of ILeVO®.

Results

Each run of this experiment was analyzed separately because the *Fusarium virguliforme* inoculum preparation differed between runs one and two and the rate of inoculum was changed between runs two and three.

SDS foliar symptom severity

For all three runs of the experiment, there were no significant main effects of cultivar or seed treatment on the SDS foliar symptoms ($P>0.05$) (Tables 3.4, 3.5, and 3.6). There was a significant interaction of pathogen and seed treatment in the first run of the experiment ($P=0.008$) (Table 3.4). Further analysis showed that for the treatments with both pathogens present, the main effects of seed treatment were significant ($P=0.026$) (Table 3.7). For run two, the interaction of seed treatment, cultivar and pathogen had a P-value of 0.043 (Table 3.5 and 3.8). Run number three showed a main effect of pathogen set on foliar symptoms ($P=<0.0001$) (Table 3.6). There was also an effect of the pathogen set by seed treatment interaction ($P=0.047$) (Table 3.6), which was no longer significant after the Tukey-Kramer adjustment was made. However, SDS foliar symptom severity was eliminated in plants infected with *F. virguliforme* and SCN compared to those only infected with SDS ($P=0.0002$) (Table 3.9). For SDS foliar symptoms, no contrasts were significant ($P>0.05$) (Tables 3.10 and 3.11).

SDS root rot severity

There was not a main effect of cultivar for any of the three runs for the root rot severity (P>0.05) (Tables 3.4, 3.5, and 3.6). For run one, there was a main effect of pathogen set (P=0.012) and the interaction of pathogen set and cultivar (P=0.005) (Table 3.4). For run one, there was a 43% reduction of root rot severity for plants infected with both SCN and SDS compared to those only infected with SDS (Table 3.9). The interaction between the pathogen set and cultivar showed a 56% reduction in root rot for plant infected with both SCN and SDS compared to plants only infected with SDS on the Pioneer 93M11 cultivar (Table 3.12). There were no significant main effects on root rot for run number two (P>0.05) (Table 3.5). Run number 3 showed a significant difference for seed treatments with regards to root rot severity (P=0.033) (Table 3.13). Root rot severity was reduced by 44% with ILeVO® in the third run for the contrast of Trilex® + Allegiance® + ILeVO® and Trilex® + Allegiance® (P=0.006) (Tables 3.11 and 3.13).

SCN Females

The main effect of cultivar was significant for runs number two and three, with significantly more SCN females, per plant and per gram of root mass, for the SCN susceptible cultivar (P<0.05) (Tables 3.5 and 3.6). The first run showed a significant main effect of seed treatment (P<0.001) (Table 3.10) and an interaction between cultivar and seed treatment (P<0.01) (Table 3.14) for both SCN females per plant and SCN females per gram of root mass.

In the first run, zero SCN females per gram of root mass were collected from plants treated with seed treatment combinations that included ILeVO®, for either contrast (P<0.001) (Table 3.11). For run number three, there were significant reductions in the numbers of SCN

females per plant and per gram of root, of 74% and 62% respectively, with ILeVO® present for the contrast of Trilex® + Allegiance® + ILeVO® and Trilex® + Allegiance® (P<0.05) (Tables 3.11 and 3.13).

SCN eggs

As with SCN females, there was a significant effect of cultivar on SCN eggs per plant and per gram of root mass for runs number two and three (P<0.05) (Tables 3.5 and 3.6). Also, run number one showed a significant effect of seed treatment (P<0.01) (Table 3.10) and an interaction between cultivar and see treatment (P<0.01) (Table 3.14) for both eggs per plant and eggs per gram of root mass.

The two contrasts for run number one were each significant for both eggs per plant and gram of root mass (P<0.01) (Table 3.11). For the Trilex® + Allegiance® + Poncho/VOTiVO® + ILeVO® and Trilex® + Allegiance® + Poncho/VOTiVO® contrast, there were reductions of 91% for eggs per plant and 90% for eggs per gram of root mass. The Trilex® + Allegiance® + ILeVO® and Trilex® + Allegiance® contrast, had reductions of 92% for eggs per plant and 91% for eggs per gram of roots mass (Table 3.10).

Root mass

Cultivar Pioneer 93Y13 had 33% more root mass than Pioneer 93M11 in run number one (P<0.001) (Table 3.15). Pathogen set reduced wet root mass in run number two by 44% (P=0.017) and run number three by 30% (P=0.01) in plants only infected with only SDS (Table 3.9).

The contrast of Trilex® + Allegiance® + ILeVO® and Trilex® + Allegiance® was significant for run number one for the wet root mass (P=0.035) (Table 3.11). There was a 23% reduction in root mass when ILeVO® was present (Table 3.10).

Root tips

The first two runs showed a significant main effect of cultivar on the number of root tips per plant (P<0.05) (Tables 3.4, 3.5). For run two, there was a main effect of pathogen set (P=0.043) and the interaction of pathogen set and cultivar (P=0.042) (Tables 3.5). For the main effect of pathogen, run two had the number of root tips was 55% lower for plants infected with *F. virguliforme* alone (Table 3.9). After sorting the data by cultivar, Pioneer 93M11 showed a 58% increase in the number of root tips present for the pathogen set with both pathogens (Table 3.16) There was also a significant effect of the pathogen set by cultivar interaction (P=0.034) for run three, but following the Tukey-Kramer adjustment, no effects were present (Table 3.6).

There was one significant contrast for the number of root tips (Table 3.11). A 41% reduction in root tips was seen for the contrast of Trilex® + Allegiance® + ILeVO® and Trilex® + Allegiance®, when ILeVO® was present, in run one(P=0.004) (Table 3.10).

Discussion

The results of this experiment suggest that under greenhouse conditions, ILeVO® does not have an effect on SDS foliar symptom severity. This is contradictory to the results attained by Mueller et al. (2011), in 2010, which showed reductions in SDS foliar symptoms severity, under field conditions, in plants treated with fluopyram. Inconsistency in effectiveness against

SDS might be attributed to difference between field and greenhouse conditions for growing the plants and fungi, including differences in inoculum density in soil.

According to Njiti et al. (2001), in an experiment performed in greenhouse settings, the rate of inoculum used in greenhouse settings had a significant effect on SDS disease severity. The rates that were used in our experiment were intended to maximize the amount of foliar symptoms that could be achieved in 35 days. However, after three runs of the experiment, average foliar symptoms severity never reached very high levels. This may be a reason why we did not see an effect of ILeVO® on SDS foliar symptoms.

Another factor that may have influenced the development of SDS foliar symptoms was the soil used. After an unsuccessful run of our experiment soil that was naturally infested with SCN was used. The use of this soil for the SDS+SCN half of the experiment may have limited, or slowed, the infection of SDS, due to the other soil micro-organisms present. This led to little to no infection by *F. virguliforme* after 35 days in that portion of the experiment. Such a reduction in SDS foliar symptoms was probably not observed in the SDS alone half due to the fact that the soil had been pasteurized to remove SCN, which in turn removed other soil micro-organisms.

The Trilex®+Allegiance®+ILeVO® combination significantly reduced root rot severity in the third run of the experiment in contrast with Trilex®+Allegiance® (Table 3.9). This, however, does not necessarily mean that the product was successful in reducing the impact of SDS. Although Luo et al. (2000) found that there was a correlation between root colonization, foliar symptoms and yield. This however is not clear evidence that root rot affects the yield of the plant in and of itself.

There may also be an effect of ILeVO® on SCN. Run one of the experiment showed significant reductions for all four response variables related to SCN in the presence of ILeVO® (Table 3.16). The numbers of SCN for this experimental run were somewhat low however. This may be explained by some work done by Gao et al. (2006), showing that in the presence of SDS, numbers of SCN were reduced. There was also a significant effect on the numbers of SCN females per plant and gram of root mass, for the Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO® compared to the Trilex®+Allegiance®+Poncho/VOTiVO® for run number three (Table 3.16). These numbers were not as low as in run one.

The root tip data from this experiment suggest that there are more root tips when SCN is present (Table 3.15). Tatalovic et al. (2013) found this same trend with regards to the presence of SCN. Tatalovic et al. (2012) also found that under adequate soil moisture conditions, *F. virguliforme* penetrates into the plants vascular tissue more frequently in the presence of SCN than it does without SCN. It has also been shown that infection of the vascular tissue allows for foliar symptom development and that penetration of roots by *F. virguliforme* occurs more frequently near the root cap (Navi and Yang, 2008). This suggests that if the number of root tips could be decreased by lowering SCN infection, SDS may be less severe as a result.

A final topic to address is the interaction of SDS and SCN. We know from several sources that SDS in the presence of SCN becomes more severe (McLean and Lawrence, 1995; Roy, 1989) and SDS foliar symptoms occur sooner, when also infected with SCN (McLean and Lawrence, 1993). Also, Gao et al. (2006) demonstrated that the presence of SDS reduces SCN infection. Our data also found that there were fewer SCN in the presence of *F. virguliforme* inoculum. However, it is unclear why the SDS foliar symptoms did not react expected. Upon

speculation, one possible reason for this is that the rate of SDS inoculum was kept relatively low to facilitate numbers of SCN. Also, the 35 day duration of the experiment was only long enough to allow one generation of SCN. It is possible that SDS foliar symptoms may have become more severe after a longer period of time.

Conclusions

ILeVO® did not have an effect on the foliar symptom severity of SDS, however it had some effect on the root rot severity. ILeVO® did affect SCN reproduction; however SCN population densities were low. In the future there are many more aspects of this study to examine. First, it would be interesting to quantify *F. virguliforme* DNA in from root samples to get a better idea of how much root infection was present in the root tissue. This method may be more accurate than visually rating the root samples. It would also be of interest to study these pathogens and these seed treatment across a range of environmental conditions. For example, the temperature could be changed across a spectrum, and the soil moisture could be altered as well. This could be done under greenhouse setting or naturally under field conditions. Another aspect that could be examined would be to look at different population densities of SCN and SDS and their reactions to ILeVO®.

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Tables and Figures

Table 3.1 Cultivar names and susceptibility to soybean cyst nematode (SCN) and soybean sudden death syndrome (SDS)

Cultivar	SCN susceptibility [z]	SDS susceptibility [y]
Pioneer 93Y13	resistant (<10% reproduction)	5
Pioneer 93M11	susceptible (>60%)	6

[y] SDS susceptibility assessed by Pioneer on a scale of 1-9; 1=poor 9=excellent
[z] SCN susceptibility is determined as the percentage of reproduction when compared to SCN reproduction on Lee 74

Table 3.2 Seed treatments used to evaluate soybean sudden death syndrome (SDS) and soybean cyst nematode (SCN) infection

Treatment number	Product combination [z]
1	red colorant (untreated control)
2	Trilex® + Allegiance®
3	Trilex® + Allegiance® + VOTiVO®
4	Trilex® + Allegiance® + Poncho®
5	Trilex® + Allegiance® + Poncho/VOTiVO®
6	Trilex® + Allegiance® + ILeVO®
7	Trilex® + Allegiance® + Poncho/VOTiVO® + ILeVO®

[z] All seed treatment combinations include red colorant

Table 3.3 Products from Bayer CropScience, active ingredient, effectiveness towards soybean cyst nematode (SCN) and soybean sudden death syndrome (SDS), rate of seed treatment applied and FRAC code

Trade name	Active Ingredient	SCN activity	SDS activity	Rate of seed treatment	FRAC code [z]
Trilex®	trifloxystrobin	no	no	5g active / 100kg of seed	11
Allegiance®	metalaxyl	no	no	4g active/ 100kg of seed	4
Poncho®	clothianidin	no	no	125g active/ 100kg of seed	
VOTiVO®	*Bacillus firmus*	yes	no	5 million units/ seed	
Poncho/VOTiVO®	clothianidin/ *Bacillus firmus*	yes	no	0.13mg active/ seed	
ILeVO®	fluopyram	unknown	yes	0.15mg active/ seed	7

[z] Fungicide resistance action committee=FRAC

Table 3.4 ANOVA summary table for the first run of the experiment evaluating the effects of ILeVO® on soybean sudden death syndrome (SDS) and the combination of SDS and soybean cyst nematode (SCN)

Source	Degrees of freedom	Sum of squares	Mean square	F value	P value
Wet root mass (g)					
Replicate	4	0.429	0.107	0.380	0.815
Seed treatment	7	2.291	0.327	1.270	0.274
Cultivar	1	4.871	4.871	18.890	<.0001
Pathogen set	1	1.028	1.028	3.640	0.127
Seed treatment x pathogen set	7	1.437	0.205	0.800	0.592
Cultivar x pathogen set	1	0.481	0.481	1.870	0.175
Seed treatment x cultivar	7	2.069	0.296	1.150	0.341
Seed treatment x cultivar x pathogen set	7	1.768	0.253	0.980	0.451
Pathogen(replication)	4	1.131	0.283	1.100	0.363
Residual	97	25.012	0.258		
AUDPC [zy]					
Replicate	4	169.733	42.433	2.380	0.211
Seed treatment	7	117.995	16.856	1.860	0.085
Cultivar	1	3.146	3.146	0.350	0.557
Pathogen set	1	89.655	89.655	5.080	0.086
Seed treatment x pathogen set	7	185.503	26.500	2.920	0.008
Cultivar x pathogen set	1	24.000	24.000	2.640	0.107
Seed treatment x cultivar	7	35.689	5.098	0.560	0.785
Seed treatment x cultivar x pathogen set	7	111.399	15.914	1.750	0.106
Pathogen(replication)	4	71.236	17.809	1.960	0.106
Residual	97	871.280	9.076		
Root rot severity (%) [z]					
Replicate	4	6.239	1.560	2.820	0.170
Seed treatment	7	2.089	0.298	1.150	0.339
Cultivar	1	0.767	0.767	2.960	0.089
Pathogen set	1	11.324	11.324	20.640	0.010
Seed treatment x pathogen set	7	1.496	0.214	0.820	0.571
Cultivar x pathogen set	1	2.180	2.180	8.400	0.005
Seed treatment x cultivar	7	1.253	0.179	0.690	0.681
Seed treatment x cultivar x pathogen set	7	2.979	0.426	1.640	0.134
Pathogen(replication)	4	2.210	0.553	2.130	0.083
Residual	97	25.448	0.260		

Table 3.4 continued

SCN females per plant [z]					
Replicate	4	77.360	19.340	8.160	<.0001
Seed treatment	7	184.143	26.306	11.100	<.0001
Cultivar	1	0.159	0.159	0.070	0.797
Seed treatment x cultivar	7	67.786	9.684	4.080	0.002
Residual	45	106.676	2.371		
SCN females per gram of root [zx]					
Replicate	4	77.143	19.286	9.600	<.0001
Seed treatment	7	160.570	22.939	11.420	<.0001
Cultivar	1	0.162	0.162	0.080	0.778
Seed treatment x cultivar	7	63.093	9.013	4.490	0.001
Residual	45	90.405	2.009		
SCN eggs per plant [z]					
Replicate	4	71.315	17.829	5.420	0.001
Seed treatment	7	114.211	16.316	4.960	0.0003
Cultivar	1	0.761	0.761	0.230	0.633
Seed treatment x cultivar	7	87.032	12.433	3.780	0.003
Residual	45	147.995	3.289		
SCN eggs per gram of root [zx]					
Replicate	4	71.702	17.926	5.990	0.001
Seed treatment	7	98.046	14.007	4.680	0.001
Cultivar	1	0.005	0.005	0.000	0.968
Seed treatment x cultivar	7	74.817	10.688	3.570	0.004
Residual	45	134.598	2.991		

Table 3.4 Continued

Number of root tips [z]					
Replicate	4	1.367	0.342	0.210	0.920
Seed treatment	7	2.703	0.386	1.550	0.160
Cultivar	1	3.029	3.029	12.160	0.001
Pathogen set	1	1.681	1.681	1.050	0.364
Seed treatment x pathogen set	7	1.803	0.258	1.030	0.413
Cultivar x pathogen set	1	0.178	0.178	0.720	0.400
Seed treatment x cultivar	7	0.966	0.138	0.550	0.791
Seed treatment x cultivar x pathogen set	7	1.153	0.165	0.660	0.705
Pathogen(replication)	4	6.519	1.630	6.540	0.0001
Residual	97	24.173	0.249		

[z] values were log transformed for statistical analyses

[y] values are the average of the value of area under disease progress curve per plant $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti+1-Ti)$

[x] values were calculated based on fresh root mass

Table 3.5 ANOVA summary table for the second run of the experiment evaluating the effects of ILeVO® on soybean sudden death syndrome (SDS) and the combination of SDS and soybean cyst nematode (SCN)

Source	Degrees of freedom	Sum of squares	Mean square	F value	P value
Wet root mass (g)					
Replicate	5	0.288	0.058	0.170	0.963
Seed treatment	7	0.662	0.095	0.940	0.478
Cultivar	1	0.230	0.230	2.290	0.134
Pathogen set	1	3.957	3.957	12.010	0.017
Seed treatment x pathogen set	7	1.352	0.193	1.930	0.076
Cultivar x pathogen set	1	0.004	0.004	0.040	0.844
Seed treatment x cultivar	7	0.121	0.017	0.170	0.990
Seed treatment x cultivar x pathogen set	7	0.513	0.073	0.730	0.646
Pathogen(replication)	5	1.692	0.339	3.380	0.008
Residual	81	8.115	0.100		
AUDPC [zy]					
Replicate	5	42.339	8.468	0.200	0.947
Seed treatment	7	13.430	1.919	0.210	0.983
Cultivar	1	1.298	1.298	0.140	0.710
Pathogen set	1	210.924	210.924	5.320	0.068
Seed treatment x pathogen set	7	84.043	12.006	1.290	0.266
Cultivar x pathogen set	1	13.206	13.206	1.420	0.237
Seed treatment x cultivar	7	117.321	16.760	1.800	0.098
Seed treatment x cultivar x pathogen set	7	142.793	20.399	2.190	0.043
Pathogen(replication)	5	207.452	41.490	4.460	0.001
Residual	81	753.626	9.304		
Root rot severity (%) [z]					
Replicate	5	2.798	0.560	0.610	0.702
Seed treatment	7	2.248	0.321	1.120	0.358
Cultivar	1	0.635	0.635	2.210	0.141
Pathogen set	1	3.111	3.111	3.510	0.118
Seed treatment x pathogen set	7	1.515	0.216	0.750	0.627
Cultivar x pathogen set	1	1.011	1.011	3.520	0.064
Seed treatment x cultivar	7	3.563	0.509	1.770	0.103
Seed treatment x cultivar x pathogen set	7	3.153	0.450	1.570	0.155
Pathogen(replication)	5	4.619	0.924	3.220	0.010
Residual	87	24.957	0.287		

Table 3.5 continued

SCN females per plant [z]					
Replicate	5	29.419	5.884	1.450	0.231
Seed treatment	7	52.797	7.542	1.860	0.107
Cultivar	1	28.384	28.384	7.000	0.012
Seed treatment x cultivar	7	39.427	5.632	1.390	0.241
Residual	35	141.914	4.055		
SCN females per gram of root [zx]					
Replicate	5	29.560	5.912	2.530	0.053
Seed treatment	7	18.103	2.586	1.110	0.388
Cultivar	1	13.538	13.538	5.790	0.023
Seed treatment x cultivar	7	27.082	3.869	1.650	0.163
Residual	27	63.142	2.339		
SCN eggs per plant [z]					
Replicate	5	28.401	5.680	1.250	0.306
Seed treatment	7	17.857	2.551	0.560	0.781
Cultivar	1	41.565	41.565	9.160	0.005
Seed treatment x cultivar	7	26.395	3.771	0.830	0.569
Residual	35	158.747	4.536		
SCN eggs per gram of root [zx]					
Replicate	5	47.904	9.581	3.170	0.023
Seed treatment	7	20.052	2.865	0.950	0.489
Cultivar	1	37.889	37.889	12.520	0.002
Seed treatment x cultivar	7	32.193	4.599	1.520	0.205
Residual	26	78.698	3.027		

Table 3.5 continued

Number of root tips [z]					
Replicate	5	11.238	2.248	1.860	0.257
Seed treatment	7	2.013	0.288	0.510	0.828
Cultivar	1	3.406	3.406	6.000	0.016
Pathogen set	1	8.232	8.232	7.000	0.043
Seed treatment x pathogen set	7	4.568	0.653	1.150	0.340
Cultivar x pathogen set	1	2.414	2.414	4.250	0.042
Seed treatment x cultivar	7	3.698	0.528	0.930	0.487
Seed treatment x cultivar x pathogen set	7	4.535	0.648	1.140	0.345
Pathogen(replication)	5	6.049	1.210	2.130	0.069
Residual	91	51.674	0.568		

[z] values were log transformed for statistical analyses

[y] values are the average of the value of area under disease progress curve per plant $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti+1-Ti)$

[x] values were calculated based on fresh root mass

Table 3.6 ANOVA summary table for the third run of the experiment evaluating the effects of ILeVO® on soybean sudden death syndrome (SDS) and the combination of SDS and soybean cyst nematode (SCN)

Source	Degrees of freedom	Sum of squares	Mean square	F value	P value
Wet root mass (g)					
Replicate	5	1.099	0.220	0.460	0.795
Seed treatment	7	0.705	0.101	0.580	0.774
Cultivar	1	0.070	0.070	0.400	0.528
Pathogen set	1	7.495	7.495	15.930	0.010
Seed treatment x pathogen set	7	0.761	0.109	0.620	0.736
Cultivar x pathogen set	1	0.000	0.000	0.000	0.991
Seed treatment x cultivar	7	1.037	0.148	0.850	0.550
Seed treatment x cultivar x pathogen set	7	0.470	0.067	0.380	0.910
Pathogen(replication)	5	2.406	0.481	2.760	0.022
Residual	106	18.516	0.175		
AUDPC [zy]					
Replicate	5	56.465	11.293	1.450	0.346
Seed treatment	7	51.444	7.349	0.640	0.721
Cultivar	1	17.249	17.249	1.500	0.223
Pathogen set	1	1096.000	1096.000	138.580	<.0001
Seed treatment x pathogen set	7	170.674	24.382	2.130	0.047
Cultivar x pathogen set	1	4.385	4.385	0.380	0.538
Seed treatment x cultivar	7	58.901	8.414	0.730	0.644
Seed treatment x cultivar x pathogen set	7	97.246	13.892	1.210	0.303
Pathogen(replication)	5	38.903	7.781	0.680	0.641
Residual	106	1215.150	11.464		
Root rot severity (%) [z]					
Replicate	5	4.969	0.994	0.660	0.673
Seed treatment	7	4.744	0.678	2.290	0.033
Cultivar	1	0.760	0.760	2.570	0.112
Pathogen set	1	0.918	0.918	0.620	0.466
Seed treatment x pathogen set	7	1.092	0.156	0.530	0.812

Table 3.6 continued

Cultivar x pathogen set	1	0.160	0.160	0.540	0.464
Seed treatment x cultivar	7	2.705	0.386	1.310	0.255
Seed treatment x cultivar x pathogen set	7	1.506	0.215	0.730	0.649
Pathogen(replication)	5	7.579	1.516	5.120	0.0003
Residual	105	31.068	0.296		
SCN females per plant [z]					
Replicate	5	9.043	1.809	1.840	0.126
Seed treatment	7	9.157	1.308	1.330	0.261
Cultivar	1	15.080	15.080	15.310	0.0003
Seed treatment x cultivar	7	8.462	1.209	1.230	0.310
Residual	42	41.357	0.985		
SCN females per gram of root [zx]					
Replicate	5	7.176	1.435	1.680	0.161
Seed treatment	7	8.321	1.189	1.390	0.234
Cultivar	1	15.549	15.549	18.200	0.0001
Seed treatment x cultivar	7	8.163	1.166	1.360	0.245
Residual	42	35.883	0.854		
SCN eggs per plant [z]					
Replicate	5	15.854	3.171	1.510	0.209
Seed treatment	7	14.683	2.098	1.000	0.448
Cultivar	1	32.764	32.764	15.550	0.0003
Seed treatment x cultivar	7	16.216	2.317	1.100	0.382
Residual	41	86.373	2.107		
SCN eggs per gram of root [zx]					
Replicate	5	13.579	2.716	1.480	0.219
Seed treatment	7	14.335	2.048	1.110	0.374
Cultivar	1	33.382	33.382	18.140	0.0001
Seed treatment x cultivar	7	15.008	2.144	1.170	0.343
Residual	41	75.436	1.840		

Table 3.6 continued

Number of root tips [z]					
Replicate	5	3.759	0.752	1.720	0.284
Seed treatment	7	0.893	0.128	0.260	0.969
Cultivar	1	0.708	0.708	1.430	0.235
Pathogen set	1	0.349	0.349	0.790	0.411
Seed treatment x pathogen set	7	1.899	0.271	0.550	0.796
Cultivar x pathogen set	1	2.297	2.297	4.640	0.034
Seed treatment x cultivar	7	4.355	0.622	1.260	0.279
Seed treatment x cultivar x pathogen set	7	2.455	0.351	0.710	0.665
Pathogen(replication)	5	2.190	0.438	0.880	0.494
Residual	105	52.005	0.495		

[z] values were log transformed for statistical analyses

[y] values are the average of the value of area under disease progress curve per plant $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti+1-Ti)$

[x] values were calculated based on fresh root mass

Table 3.7 Tukey-Kramer estimates of all seed treatments, sorted by pathogen, on mean soybean sudden death syndrome (SDS) foliar symptom severity for the first run of the experiment evaluating the effects of ILeVO® averaged across all pathogen sets

Seed treatment	SDS foliar symptoms SDS alone [zyx]	SDS foliar symptoms SCN+SDS [zyx]
naked	10 a	15 ab
Red colorant	59 a	0 b
Trilex®+Allegiance®	58 a	1 ab
Trilex®+Allegiance®+VOTiVO®	6 a	3 ab
Trilex®+Allegiance®+Poncho®	5 a	20 ab
Trilex®+Allegiance®+Poncho/VOTiVO®	51 a	5 ab
Trilex®+Allegiance®+ILeVO®	53 a	29 ab
Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO®	61 a	33 a

[z] values were log transformed for statistical analyses and back transformed to present in tables

[y] estimates with different letters are statistically different (P<0.05)

[x] values are the average of the value of area under disease progress curve per plant $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti+1-Ti)$

Table 3.8 Tukey-Kramer estimates for main effect of all seed treatments on mean response variables, for run#2 of the experiment evaluating the effects of ILeVO® averaged across all pathogen sets

Seed treatment	Wet root mass (g) [y]	SCN females per plant [zy]	SCN eggs per plant [zy]	SCN females per gram of root [zyx]	SCN eggs per gram of root [zyx]	SDS foliar symptoms [zyw]	SDS root rot severity (%) [zy]	Root tips [zy]
naked	1.09 a	7 a	610 a	7 a	497 a	42 a	56 a	322 a
Red colorant	1.02 a	6 a	488 a	10 a	708 a	37 a	44 a	387 a
Trilex®+Allegiance®	1.17 a	10 a	402 a	8 a	402 a	48 a	53 a	367 a
Trilex®+Allegiance®+VOTiVO®	0.97 a	1 a	106 a	2 a	106 a	39 a	49 a	301 a
Trilex®+Allegiance®+Poncho®	0.93 a	6 a	413 a	4 a	413 a	36 a	59 a	432 a
Trilex®+Allegiance®+Poncho/VOTiVO®	0.96 a	1 a	161 a	1 a	161 a	30 a	50 a	330 a
Trilex®+Allegiance®+ILeVO®	1.07 a	2 a	149 a	3 a	149 a	10 a	46 a	357 a
Trilex®+Allegiance®+Poncho/VOTiVO®+ ILeVO®	1.11 a	3 a	323 a	6 a	323 a	44 a	36 a	475 a

[z] values were log transformed for statistical analyses and back transformed to present in tables

[y] estimates with different letters are statistically different ($P<0.05$)

[x] values were calculated based on fresh root mass

[w] values are the average of the value of area under disease progress curve per plant $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti+1-Ti)$

Table 3.9 Tukey-Kramer estimates of each pathogen set on mean response variables, for all three runs of the experiment evaluating the effects of ILeVO® averaged across all pathogen sets

Run	Pathogen set	Wet root mass (g) [y]	SDS foliar symptoms [zyx]	SDS root rot severity (%) [zy]	Root tips [zy]
1	SDS alone	1.62 a	26 a	37 a	372 a
1	SDS+SCN	1.44 a	5 a	21 b	468 a
2	SDS alone	0.83 b	158 a	58 a	272 b
2	SDS+SCN	1.25 a	7 a	41 a	497 a
3	SDS alone	1.14 b	54 a	27 a	555 a
3	SDS+SCN	1.62 a	0 b	23 a	615 a

[z] values were log transformed for statistical analyses and back transformed to present in tables
[y] estimates with different letters are statistically different (P<0.05)
[x] values are the average of the value of area under disease progress curve per plant $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti+1-Ti)$

Table 3.10 Tukey-Kramer estimates for main effect of all seed treatments on mean response variables, for run#1 of the experiment evaluating the effects of ILeVO® averaged across all pathogen sets

Seed treatment	Wet root mass (g) [y]	SCN females per plant [zy]	SCN eggs per plant [zy]	SCN females per gram of root [zyx]	SCN eggs per gram of root [zyx]	SDS foliar symptoms [zyw]	SDS root rot severity (%) [zy]	Root tips [zy]
naked	1.65 a	6 a	659 a	5 a	472 a	12 a	35 a	426 a
Red colorant	1.45 a	5 a	504 a	4 a	428 a	2 a	26 a	478 a
Trilex®+ Allegiance®	1.70 a	8 a	573 a	5 a	372 a	7 a	27 a	522 a
Trilex®+ Allegiance®+VOTiVO®	1.69 a	11 a	589 a	6 a	335 a	4 a	31 a	418 a
Trilex®+ Allegiance®+Poncho®	1.56 a	7 a	312 a	5 a	197 a	10 a	26 a	425 a
Trilex®+ Allegiance® +Poncho/VOTiVO®	1.48 a	3 a	181 ab	2 a	146 ab	16 a	26 a	377 a
Trilex®+ Allegiance®+ILeVO®	1.31 a	0 b	40 ab	0 b	35 ab	37 a	28 a	306 a
Trilex®+ Allegiance®+Poncho/VOTiVO®+ ILeVO®	1.41 a	0 b	16 b	0 b	14 b	44 a	22 a	420 a

[z] values were log transformed for statistical analyses and back transformed to present in tables

[y] estimates with different letters are statistically different (P<0.05)

[x] values were calculated based on fresh root mass

[w] values are the average of the value of area under disease progress curve per plant $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti+1-Ti)$

Table 3.11 P-values for contrasts between seed treatment combinations with and without ILeVO® across all response variables for three separate runs of the experiment averaged across all pathogen sets

Run	Contrast	Wet root mass (g)	SCN females per plant [z]	SCN eggs per plant [z]	SCN females per gram of root [zx]	SCN eggs per gram of root [zx]	SDS foliar symptoms [zy]	SDS root rot severity (%) [z]	Root tips [z]
1	Trilex®+Allegiance®+Poncho/VOTiVO® x Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO®	0.666	<0.0001	0.006	<0.0001	0.005	0.311	0.921	0.519
1	Trilex®+Allegiance® x Trilex®+Allegiance®+ILeVO®	0.035	<0.0001	0.007	<0.0001	0.013	0.113	0.398	0.004
2	Trilex®+Allegiance®+Poncho/VOTiVO® x Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO®	0.211	0.352	0.570	0.119	0.070	0.746	0.079	0.179
2	Trilex®+Allegiance® x Trilex®+Allegiance®+ILeVO®	0.420	0.200	0.432	0.351	0.516	0.246	0.456	0.921
3	Trilex®+Allegiance®+Poncho/VOTiVO® x Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO®	0.478	0.316	0.612	0.221	0.517	0.353	0.766	0.966
3	Trilex®+Allegiance® x Trilex®+Allegiance®+ILeVO®	0.422	0.022	0.189	0.027	0.211	0.852	0.006	0.835

Table 3.11 continued

[z] values were log transformed for statistical analyses

[y] values are the average of the value of area under disease progress curve per plant $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti + 1 - Ti)$

[x] values were calculated based on fresh root mass

Table 3.12 Tukey-Kramer estimates of the effects of Pathogen set, sorted by cultivar, on mean soybean sudden death syndrome (SDS) root rot severity, for run #1 of the experiment evaluating the effects of ILeVO® averaged across all pathogen sets

Cultivar	SDS root rot severity (%) Pioneer 93Y13 [zyx]	SDS root rot severity (%) Pioneer 93M11 [zyx]
SDS alone	35 a	39 a
SDS+SCN	25 a	17 b

[z] values were log transformed for statistical analyses and back transformed to present in tables
[y] estimates with different letters are statistically different ($P<0.05$)
[x] values were log transformed for statistical analyses and back transformed to present in tables

Table 3.13 Tukey-Kramer estimates for main effect of all seed treatments on mean response variables, for run #3 of the experiment evaluating the effects of ILeVO® averaged across all pathogen sets

Seed treatment	Wet root mass (g) [y]	SCN females per plant [zy]	SCN eggs per plant [zy]	SCN females per gram of root [zyx]	SCN eggs per gram of root [zyx]	SDS foliar symptoms [zyw]	SDS root rot severity (%) [zy]	Root tips [zy]
naked	1.57 a	14 a	335 a	8 a	182 a	1 a	31 a	614 a
Red colorant	1.31 a	20 a	1466 a	12 a	921 a	9 a	25 a	568 a
Trilex®+Allegiance®	1.46 a	34 a	2133 a	21 a	1318 a	2 a	30 a	586 a
Trilex®+Allegiance®+VOTiVO®	1.42 a	27 a	2176 a	18 a	1394 a	3 a	30 a	494a
Trilex®+Allegiance®+Poncho®	1.34 a	23 a	2176 a	14 a	1354 a	4 a	27 a	578 a
Trilex®+Allegiance®+Poncho/VOTiVO®	1.24 a	22 a	1367 a	16 a	998 a	2 a	20 a	616 a
Trilex®+Allegiance®+ILeVO®	1.34 a	9 a	715 a	6 a	498 a	3 a	17 a	618 a
Trilex®+Allegiance®+Poncho/VOTiVO® +ILeVO®	1.35 a	13 a	948 a	9 a	646 a	5 a	21 a	610 a

[z] values were log transformed for statistical analyses and back transformed to present in tables

[y] estimates with different letters are statistically different ($P<0.05$)

[x] values were calculated based on fresh root mass

[w] values are the average of the value of area under disease progress curve per plant $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti+1-Ti)$

Table 3.14 Tukey-Kramer estimates of all seed treatments, sorted by cultivar, on mean soybean cyst nematode (SCN) eggs and females, for run #1 of the experiment evaluating the effects of ILeVO® averaged across all pathogen sets

Seed treatment	SCN eggs per plant Pioneer 93M11 [zy]	SCN eggs per gram of root Pioneer 93M11 [zyx]	SCN females per plant Pioneer 93M11 [zy]	SCN females per gram of root Pioneer 93M11 [zyx]	SCN eggs per plant Pioneer 93Y13 [zy]	SCN eggs per gram of root Pioneer 93Y13 [zyx]	SCN females per plant Pioneer 93Y13 [zy]	SCN females per gram of root Pioneer 93Y13 [zyx]
naked	451 a	378 a	7 ab	6 ab	1233 a	734 a	6 a	3 a
Red colorant	809 a	745 a	10 ab	9 a	342 a	261 a	2 a	2 ab
Trilex®+Allegiance®	636 a	433 a	10 ab	7 ab	330 a	216 a	6 a	4 a
Trilex®+Allegiance®+VOTiVO®	2412 a	1309 a	27 a	15 a	153 a	87 a	5 a	3 a
Trilex®+Allegiance®+Poncho®	1060 a	645 a	14 a	9 a	95 a	60 a	4 a	3 a
Trilex®+Allegiance®+Poncho/VOTiVO®	90 ab	100 ab	1 abc	2 ab	335 a	198 a	6 a	4 a
Trilex®+Allegiance®+ILeVO®	6 b	6 b	0 bc	0 bc	355 a	281 a	3 ab	2 a
Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO®	4 b	4 b	0 c	0 c	72 a	46 a	0 b	0 b

[z] values were log transformed for statistical analyses and back transformed to present in tables

[y] estimates with different letters are statistically different ($P<0.05$)

[x] values were calculated based on fresh root mass

Table 3.15 Tukey-Kramer estimates of each cultivar on mean response variables, for all three runs of the experiment evaluating the effects of ILeVO® averaged across all pathogen sets

Run	Cultivar	Wet root mass (g) [y]	SCN females per plant [zy]	SCN eggs per plant [zy]	SCN females per gram of root [zyx]	SCN eggs per gram of root [zyx]	SDS foliar symptoms [zyw]	SDS root rot severity (%) [zy]	Root tips [zy]
1	Pioneer 93Y13	1.73 a	3 a	238 a	2 a	157 a	13 a	30 a	487 a
1	Pioneer 93M11	1.34 b	2 a	190 a	2 a	154 a	9 a	25 a	357 b
2	Pioneer 93Y13	0.99 a	1 b	110 b	2 b	118 b	31 a	53 a	196 b
2	Pioneer 93M11	1.09 a	7 a	726 a	8 a	852 a	35 a	45 a	440 a
3	Pioneer 93Y13	1.45 a	11 b	540 b	7 b	344 b	4 a	27 a	549 a
3	Pioneer 93M11	2.26 a	33 a	2713 a	21 a	1757 a	2 a	23 a	621 a

[z] values were log transformed for statistical analyses and back transformed to present in tables

[y] Estimates with different letters are statistically different ($P<0.05$)

[x] values were calculated based on fresh root mass

[w] values are the average of the value of area under disease progress curve per plant $AUDPC = \sum_{i=1}^{n-1} \frac{Yi+Yi-1}{2} x(Ti+1-Ti)$

Table 3.16 Tukey-Kramer estimates of the effects of Pathogen set, sorted by cultivar, on mean root tips per plant, for run #2 of the experiment evaluating the effects of ILeVO® averaged across all pathogen sets

Cultivar	Root tips Pioneer 93Y13 [zy]	Root tips Pioneer 93M11 [zy]
SDS alone	203 b	361 a
SDS+SCN	481 a	531 a

[z] values were log transformed for statistical analyses and back transformed to present in tables
[y] Estimates with different letters are statistically different ($P<0.05$)

CHAPTER 4. SUMMARY

General Conclusions

ILeVO® did not have an effect on SDS foliar symptom severity for our research. There was a slight effect, however, on the root rot severity caused by *F. virguliforme*. ILeVO® had a consistent effect on SCN reproduction when SCN was alone and in in the presence of *F. virguliforme*.

Future Research

There are several areas of research that could stem from this study. One of these areas of further research would be to look at ILeVO® on its own with no other products. Other studies could also examine the effects of ILeVO® on SDS or SCN under different environmental conditions such as temperature. One final study could look at the effects of ILeVO® on different populations of SDS or SCN

APPENDIX A. PLANT SIZE IN RESPONSE TO ILeVO®

After the first experiment looking at soybean cyst nematode (SCN) alone was completed, a significant effect of seed treatment on root mass was observed. This prompted a study looking at how each of the seed treatment combinations affected plant size, without any pressure from a pathogen.

Materials and Methods

Two runs of an experiment were performed. Each of the four cultivars (Table 2.1), with each of the seven seed treatments (Table 2.3), was planted into pasteurized soil. Each run had six replicates. Plants were grown for 30 days in water baths set to 27˚C. At the end of the 30-day period, wet and dry masses were recorded for the plant tops and plant roots (dry mass data not shown).

Results

When this experiment was analyzed there was a significant main effect of seed treatment on root mass (Table A.1). Contrasts were made for the pairs of seed treatment with and without ILeVO®. The contrasts show that ILeVO® did not have an effect on wet root mass for either pairing (Table A.2). There was, however, a significant effect of treatment on wet top mass, and the contrast for of Trilex®+Allegiance®+ILeVO® and Trilex®+Allegiance®, showed a significant reduction of mass in the presence of ILeVO® (P=0.035) (Table A.2).

Tables and Figures

Table A.1 LSmeans for the main effect of seed treatment, across all response variables for both separate runs of the experiment examining the effects of ILeVO® on plant mass

Seed treatment	Wet root mass (g) [y]	Wet top mass (g) [y]
naked	2.80 a	3.37 a
Red colorant	2.60 ab	3.31 ab
Trilex®+ Allegiance®	2.62 ab	3.2 abc
Trilex®+ Allegiance®+VOTiVO®	2.78 a	3.05 abc
Trilex®+ Allegiance®+Poncho®	2.30 b	2.74 c
Trilex®+ Allegiance® +Poncho/VOTiVO®	2.31 b	2.92 abc
Trilex®+ Allegiance®+ILeVO®	2.48 ab	2.81 bc
Trilex®+ Allegiance®+Poncho/VOTiVO®+ ILeVO®	2.31 b	2.81 c

[y] Means with different letters are statistically different (P<0.05)

Table A.2 Contrasts between seed treatment combinations with and without ILeVO® across all response variables for both separate runs of the experiment examining the effects of ILeVO® on plant mass

Contrast	Wet root mass (g)	Wet top mass (g)
Trilex®+Allegiance®+Poncho/VOTiVO® x Trilex®+Allegiance®+Poncho/VOTiVO®+ILeVO®	0.990	0.525
Trilex®+Allegiance® x Trilex®+Allegiance®+ILeVO®	0.326	0.035

APPENDIX B. EFFECTS OF GROWTH SUBSTRATE ON *Fusarium virguliforme* AND SOYBEAN CYST NEMATODE

This experiment was performed to evaluate four types of substrate for their ability to culture *Fusarium virguliforme,* such that good soybean sudden death syndrome (SDS) foliar symptoms would occur, and allow soybean cyst nematode (SCN) to infect plant roots.

Materials and Methods

Experiments were performed in a growth chamber set to 24ºC and 16 hour light duration. Plants were grown in individual cone-tainers and placed into bucket filled with sand to maintain similarity to water bath experiments.

The four substrates used were cornmeal, cracked corn, ground sorghum, and whole sorghum. For the cornmeal and ground sorghum, the same procedure outlined by Munkvold et al. (2002) was used, 400 ml of cornmeal, 1,900 ml of sand, 100 ml of water and 2 ml of spore suspension (1,000,000 spores/ml). This procedure was then adapted for the whole cracked corn and the whole sorghum. For these two substrates, no sand was added to the mixture. Instead 2,300 cm^3 of the substrate and 100 ml of water was added to an autoclave bag and 2 ml of spore suspension. This was done to ensure easier dispersal of the fungus to all of the grains. For each of the four substrates, a control substrate was made, which was not inoculated with *Fusarium virguliforme*.

Each type of substrate was then diluted into a pasteurized sand-soil mixture, at rates of 1:30 and 1:50, inoculum: sand/soil mixture. Once the substrate was diluted, the control substrate sand-soil mixtures which were not inoculated with *F. virguliforme* were inoculated with SCN.

SCN was inoculated by adding females that had been sprayed from roots of culture plants. Approximately 200 SCN females were added to each aliquot of soil mixed with substrate. This would show the effects of the substrate on the ability of SCN to infect plants.

Four replicates of each substrate combination were grown for each pathogen. Plants were grown for 35 days to allow SCN to complete one generation. During the 35 day period, plants were rated every other day for SDS foliar symptom severity. At the end of the thirty days, SDS root rot severity was measured and female SCN were collected from roots. SCN females were then counted.

Results

After processing the data, we decided to use the whole sorghum substrate at a rate of 1:30. Though the number of SCN present for this substrate was lowest, the SDS foliar symptoms expressed on plants inoculated with sorghum inoculum far exceeded the other types of substrate (Table 1). This substrate and inoculum was used to test the effects of ILeVO® on SDS alone and SDS with SCN.

Literature Cited

1. Munkvold, G. P., and O'Mara, J. K. 2002. Laboratory and growth chamber evaluation of fungicidal seed treatments for maize seedling blight caused by *Fusarium* species. Plant Disease 86:143-150.

Tables and Figures

Table B.1 LSmeans for the main effects of inoculum substrate, separated by pathogen for the study examining the most effective substrate to culture *Fusarium virguliforme* and allow soybean cyst nematode (SCN) infection, performed in a growth chamber set to 24˚C

Substrate	SCN females per plant [yx]	Substrate	SDS foliar symptom severity [yx]
No substrate + SCN	169 a	1:10 cornmeal	174 b
1:30 cornmeal	81 cd	1:30 cornmeal	99 b
1:30 cracked corn	113 bc	1:30 cracked corn	121 b
1:30 ground sorghum	108 c	1:30 ground sorghum	116 b
1:30 whole sorghum	63 d	1:30 whole sorghum	1,053 a
1:50 cornmeal	141 ab	1:50 cornmeal	239 b
1:50 cracked corn	93 cd	1:50 cracked corn	165 b
1:50 ground sorghum	83 cd	1:50 ground sorghum	61 b
1:50 whole sorghum	83 cd	1:50 whole sorghum	950 a
No substrate or SCN	4 e	No substrate or SDS	46 b

[y] Means with different letters are statistically different ($P<0.05$)

[x] values are the average of the value of area under disease progress curve per plant

APPENDIX C. EFFECTS OF GREENHOUSE CONDITIONS ON CULTIVAR RATINGS

To ensure the ratings for soybean sudden death syndrome (SDS) susceptibility provided by Asgrow and Pioneer were not different under greenhouse conditions; this experiment was conducted looking at the effects of SDS on each of the four seed cultivars (Table 2.1) without any seed treatment.

Materials and Methods

Plants were grown in cone-tainers, which were placed in a bucket filled with sand, then placed in a water bath at 24˚C. Plants were then allowed to grow for 30 days. To establish a baseline of SDS resistance and susceptibility, two known resistant and two known susceptible cultivars were selected. For SDS resistant cultivars we selected Jack and MN1606. For SDS susceptible cultivars we selected Williams 82 and MAC02. Once the first SDS foliar symptoms were observed, ratings as a percentage of leaf area affected were taken every other day. At the end of 30 days, root rot severity was taken as a percentage.

Results

There was not a significant difference in SDS susceptibility, for any of our four selected varieties for either foliar symptom severity or root rot severity (Table B.1).

Tables and Figures

Table C.1 LSmeans for the main effects of cultivar, for the study examining soybean sudden death syndrome (SDS) susceptibility in 4 soybean cultivars, performed in a water bath set to 24˚C

Cultivar	SDS foliar symptom severity [zy]	Root rot (%) [z]
Jack [x]	257 bc	58 a
MN1606 [x]	23 c	60 a
Pioneer 93Y13	547 abc	53 a
Asgrow AG 3231	1136 a	61 a
Asgrow AG 3432	752 ab	62 a
Pioneer 93M11	339 bc	65 a
Williams 82 [w]	797 ab	71 a
MAC02 [w]	887 ab	80 a

[z] Means with different letters are statistically different ($P<0.05$)
[y] values are the average of the value of area under disease progress curve per plant
[x] Soybean cultivars with resistance to SDS
[w] Soybean cultivars those are susceptible to SDS

APPENDIX D. EFFECTS OF TEMPERATURE ON SOYBEAN CYST NEMATODE AND LIFE CYCLE DEVELOPMENT

To ensure that SCN had adequate time to complete one generation in 35 days at 24˚C, an experiment was performed looking at the numbers of SCN at six different time points.

Materials and Methods

The SCN susceptible cultivar Pioneer 93M11 was planted into Oskaloosa Iowa soil naturally infested with SCN. The Initial egg count for this soil was 4,200 eggs/100cm^3 of soil. Cone-tainers were labeled for one of six time points: 27, 30, 33, 36, 39, and 42 days. Six replicates were randomly arranged in buckets of sand, and then placed in a water bath at 24˚C. Plants were processed on the days that they were assigned.

At each time point, plant roots were sprayed to collect SCN females. Wet and dry root mass was measured for each plant. Females were counted then later processed to count numbers of eggs.

Results

The number of SCN females peaked at 39 days after planting at 24˚C (Table D.1). Also, the number of eggs per female continued to increase all the way to 42 days even after the number of SCN females began to decline (Table D.1).

Tables and Figures

Table D.1 LSmeans for the main effects of number of days, for the study of the effect on duration of plant growth on soybean cyst nematode (SCN) population densities, performed in a water bath set to 24˚C

Days	SCN females per gram of wet root mass [y]	SCN eggs per female [yx]
27	29 c	18 c
30	66 c	35 c
33	83 c	55 bc
36	140 b	92 b
39	251 a	150 a
42	170 b	185 a

[y] Means with different letters are statistically different ($P<0.05$)

[x] values are the quotient of SCN eggs divided by SCN females per plant