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Effect of infection site and glyphosate application on the foliar symptoms expression of soybean sudden death syndrome

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CHAPTER 1. GENERAL INTRODUCTION

Thesis organization

This thesis is organized in 4 chapters. The first chapter includes a review of literature and a justification for the research reported in this thesis. The second chapter is a study of the effect of root infection sites and physical injury on the development of foliar symptoms of sudden death syndrome (SDS). The third chapter is a study of the effect of glyphosate on the development of SDS foliar symptoms in 4 different soybean cultivars. The last chapter is a summary and general conclusion of the studies described in this thesis.

Literature Review

History, importance, and distribution of SDS

Sudden death syndrome is an important disease of soybean [*Glycine max* (L) Merr.] caused by different species of *Fusarium* (11, 20, 21, 27). The disease was first observed in Arkansas in the early 70s by H. J. Walters (34), but the disease was not given much attention until a decade later when considerable yield losses were observed in that state due to SDS. In 1984, the disease was also documented in four other states: Mississippi, Missouri, Tennessee and Kentucky. Now SDS is found in all major soybean growing regions in the US, South America (4, 5, 30, 38, 43) and Canada (1). *F. cuneirostrum*, a SDS-causing fungus in South America, has been isolated from roots of dry beans (*P. vulgaris*) in Japan but no SDS symptoms have been reported in that country (4).

Symptomatic plants have reduced yield and seed quality (26). In field conditions, there are reports of 80% yield reduction due to SDS, but 10-20% yield losses are more common. In spite of the fact the disease causes necrosis on soybean leaves, the pathogen has not been isolated on the aerial parts of the symptomatic plants (24, 34).

In Argentina, SDS was first observed in fields of the Pampas Region during the growing season of 1991, and in the following year on fields of the Northwest Region (39). In the seasons following its detection, SDS gradually increased its importance and became one of the most important diseases on soybeans in Argentina (42). In that country, SDS caused losses of 134,000 metric tons (43) in 1994, 147,000 metric tons in 1998 (46) and 769.3 metric tons in 2006 (42).

Sudden death syndrome of soybean (SDS) was first observed in Brazil during the growing season of 1981 at São Gotardo, Minas Gerais state (50). The characterization of its causal agent and fulfillment of Koch's postulates was not done until 1996 (23). The losses due to SDS in Brazil in 1994 were estimated in 15,000 metric tons (43), 143,000 in 1997 and 200,000 in 1998 (48, 49). During the growing season of 1999, SDS was documented in 99 municipalities in 8 states (BA, GO, MT, MS, PR, RS, and SP), corresponding to an area of 2 million hectares. Yorinori estimated losses of 53 million dollars due SDS on that year (49). In 2006 losses due to SDS in Brazil were 320,000 metric tons (42).

SDS causal agents

When the disease was first reported in Arkansas in 1972 (35) its causal agent was designated as *Fusarium solani*. This designation remained until 1997 when Roy reported that a morphologically distinguished form of *F. solani* was responsible for SDS symptoms in soybean. At this point, the SDS causal organism was designated as *F. solani* f. sp. *glycines* (33). But in

2003 Aoki *et al*. reported two phylogenetically and morphologically distinct species of *Fusarium* that cause SDS, *Fusarium virguliforme*, formally known as *F. solani* f. sp. *glycines*, and *F. tucumaniae*, the first was proposed as the causal agent in North America and the latter for South America (2). currently it is known that SDS is caused by four different Fusarium species in South America, *F. brasiliense*, *F. cuneirostrum, F. virguliforme*, and *F. tucumaniae*, the later has been the most important causal agent in that region, whereas in North America *F.* v*irguliforme* is the only causal agent reported for this disease (3, 27).

Symptomatology

The fungi colonize the roots of plants in the field, generally causing external root rot and internal reddish to brown discoloration of the taproot and the base of the stem (8). Root rot severity can vary from necrotic spots on the tap root to total destruction of lateral roots. Foliar symptoms are observed mostly after flowering but they can be seen as early as 2 weeks after planting (12). Leaves show interveinal chlorosis, initially with clear small tissue patches in between the veins that may coalesce forming larger patches of chlorotic tissue that might become necrotic. In later stages, leaves with severe symptoms eventually are detached from the plant at the base of the petiole (34, 47). There have been studies showing that root dry weight and root rot severity are correlated to severity of foliar symptoms of SDS (18, 31).

The foliar symptoms are caused by the translocation, via xylem, of toxin produced by the pathogen while colonizing the roots (12, 16, 17). So far, only one toxin produced by *F. virguliforme* has been identified as responsible for inducing expression of foliar symptoms. It has been characterized as a protein of 17kDa of mass (16). The toxin can induce SDS foliar

symptoms after being absorbed by the roots of soybean plants in the absence of the pathogen (13, 19, 40).

Epidemiology and management

F. virguliforme can survive harsh environmental conditions by forming chlamydospores intercalary in hyphae or by modifying macroconidial cells (1, 34). This survival structure is considered the primary inoculum of the disease (12). The pathogen appears to penetrate the roots directly through the epidermis during the infection process (24, 34). Increased conidial germination and root penetration have been reported when conidia are located at the base of root hairs or at the root cap (41). In green house experiments, early infection has been shown to be a key factor for the development of foliar symptoms of SDS; plants did not show symptoms when inoculated 15 days after planting or later (47). After infection, colonization can be restricted to cortical tissues, but research shows that foliar symptoms are well correlated to the colonization of xylem by the fungi (24). At the end of a growing season, masses of conidia and chlamydospores can be observed in rotted roots and sloughed radicular tissue of soybean plants under field conditions (32).

Green house studies have found few soybean genotypes to be qualitatively resistant to SDS, but in field conditions only quantitative resistance has been documented on the same genotypes (9,10, 51). Nevertheless, the use of horizontally resistant cultivars is recommended for the control of SDS (8).

Planting date has been shown to be relevant to the early onset of the disease. Early planting results in the plants in a cooler environment, due to low soil temperature in US soybean growing regions in the early spring, which is favorable to root infection (15, 22, 45). Hence,

delaying planting is an escape strategy recommended to decrease the risk of foliar symptom development (8).

Field studies conducted in Argentina under natural infestation associate sudden death syndrome of soybean with no-till cropping systems and increased disease parameters were observed in plots rotated with corn (30). The increase of SDS severity in no-till cropping system was also reported earlier in the US (45) and observed in Brazil (28). This effect is probably related to the fact that tilled soil generally has lower moisture levels in upper layers and it is warmer than no-till (29).

Water management such as adequate irrigation and practices that improve drainage of potential saturated soil are also recommended for the control of SDS since the development of foliar symptoms is favored by high moisture or saturated soil conditions (8, 12).

Glyphosate and soybean sudden death syndrome

Glyphosate [N-(phosphonomethyl) glycine] is a post emergent broad spectrum non selective herbicide (51). Since the release of glyphosate-tolerant soybean cultivars, the usage of this herbicide has greatly increased in all soybean producing regions. Glyphosate has been shown to increase disease levels in selected pathosystems (9). Increased SDS levels were observed by Sanogo *et al*. in soybean plants when glyphosate was applied (37). Njiti evaluated the effect of glyphosate on SDS disease parameters on different cultivars of soybean but was unable to detect any effect (25).

After the release of glyphosate-resistant soybean cultivars in 1996, there was a progressive replacement of many herbicides used on soybean with glyphosate in the soybean production (6). In 1994, glyphosate was used in 15% of the US soybean acreage; the use

increased to over 60% and 95% in 2000 and 2006, respectively. In the year 2000, glyphosate was the most widely used herbicide in the world (14). It also has been reported that the number of glyphosate applications per growing season has increased in soybean fields in the US since the release of glyphosate tolerant cultivars due to the development of resistance by weeds (6).

In 2002, on soybean fields planted with glyphosate resistant cultivars, no-tillage cropping system acreage was almost 5 fold greater than conventional tillage, over 9.5 million and slightly over 2 million ha, respectively, whereas in areas of non-transgenic soybean, the two cropping system had similar acreage (7). SDS has been reported with increased incidence and severity on no-till fields (28, 30, 45) which could aggravate the problem.

Means and Kremer have shown that glyphosate resistant soybean plants, grown in green house conditions in farm soil, had higher number of *Fusarium* colony forming units on their roots when plants were sprayed with glyphosate compared to non-sprayed; the difference was greater in high soil moisture (21). These results were in agreement with previous work done by Sanogo *et al*. (37). Kremer showed that after application of glyphosate on resistant and susceptible soybean plants, both had significant increases in the quantities of carbohydrate and amino acids secreted by their roots (20). Measurements of enzymatic activity and respiration rate on soil recovered from rhizosphere of glyphosate resistant soybean plants indicate that the application of glyphosate on those plants increased the microbiological activity on their rhizosphere under optimum field conditions (21).

Justification

During the past decade, SDS has become the top 10 diseases that cause yield reduction in soybean grown in South and North America (42-44, 46, 48, 49). Although diverse cultural practices are known to decrease disease levels, such as adjusting planting date and the use of resistant genotypes, they are not consistent .The soybean - *F. virguliforme* pathosystem has been studied for over 40 years, generating a partial understanding of the disease process but many questions remain unanswered.

In this study, our objective was to investigate two factors that affect the development of SDS foliar symptoms, one factor being from host itself and one being an external factor. In a previous work done by Navi and Yang in greenhouse experiments, it was shown that germination and infection of *F. virguliforme* macroconidia was influenced by its location on soybean roots. Based on their observations, we proposed that the root site that interface with the pathogen propagules can play a role in whether or not foliar symptoms are expressed in result of that infection. Our first objective was to evaluate the development of SDS foliar symptoms when different root sites were inoculated with *F. virguliforme* macroconidia.

For the second objective, there have been reports that glyphosate application affects the severity of SDS foliar symptom (36). Sanogo reports that glyphosate increased foliar symptoms of SDS in green house and field conditions (36). However, Njiti reports that the application of glyphosate has no effect on SDS with field data from one growing season (25), which is conflicting with Sanogo *et al*. finding. These studies were done in the early years after introduction of Roundup Ready® soybean. Our objective was to evaluate the effect of glyphosate on the development of foliar symptoms of SDS.

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CHAPTER 2. EFFECT OF INOCULATION SITES AND PHYSICAL INJURY ON FOLIAR SYMPTOMS EXPRESSION

Abstract

Soybean sudden death syndrome (SDS), caused by *Fusarium virguliforme*, is an important root disease that can cause root rot and express foliar symptoms leading to premature defoliation and yield reduction. Earlier reports suggested that the germination of *F. virguliforme* macroconidia and penetration of their germination tube may differ among the sites along the root system of soybean plants. The objective of this study was to assess effects of different root inoculation sites on foliar symptom expression and the effect of mechanical injury of seedling tap roots on foliar symptoms expression of SDS. We evaluated three different sites of infection: root tip, middle root portion and upper root. After inoculation, seedlings were transplanted to cones filled with sterile potting mixture. Rhizosphere temperature was controlled by using a water bath (20°C). Plants were evaluated three weeks after transplanting for incidence and severity of SDS. Root sites were not significant for incidence of SDS. Higher incidence was observed when root tips were wounded, probably because tissue wound increased xylem infections. A field survey was done to compare disease levels for plants grown in greenhouse to plants in the fields. Adjacent symptomatic and asymptomatic plants were collect from three fields naturally infested with *F. virguliforme.* Our results suggested that *F. virguliforme* can infect plants and remain asymptomatic at a high incidence level.

Introduction

Sudden death syndrome (SDS) is a major root rot disease on soybean in all production regions in America (20, 23, 30, 34). It is caused by *Fusarium virguliforme* in North America and at least five other *Fusarium* species in South America (1-3, 17). While colonizing the root system, the fungi produce toxins that are transported upwards via the xylem and cause symptoms on leaves. Initial symptoms are small chlorotic spots on younger leaves, spots may coalesce and form larger chlorotic spots that may evolve to necrosis of the affected foliar tissue, leading to great yield reduction in severe cases (20). In isolated fields, losses of 80% have been reported but yield reductions of 15-20% are more common (4, 20).

The effect of environment and cultural practices such as planting date (11, 22, 31), crop rotation (18, 32), genetic resistance (12, 25, 29, 33), temperature (10, 24), irrigation (6, 14), and isolate aggressiveness (13, 21) on SDS have been studied for the past 30 years, since the disease was first report in Arkansas. Although great progress has been made toward understanding the mechanisms of sudden death syndrome development, this pathosystem is not yet completely understood, which makes SDS outbreaks hard to predict or explain in detail. Disease severity and incidence occur with great variability, even among plants from the same field and genetic background.

Plant roots include regions with different cellular development stages (27); namely, meristematic region, postmitotic region, elongation zone, and maturation zone. Specific root regions can be particularly susceptible to infection by plant pathogenic fungi, for instance *Nectria haematococca*, a teleomorphic state of *F. solani*, infects pea roots more successfully through the elongation zone (7, 8), whereas *Fusarium oxysporum* colonizes cotton roots more

efficiently through the meristematic region (19), but is not site-dependent when infecting tomato roots (15).

Earlier reports suggested that leaf symptom expression of SDS may have been affected by the location on the root where infection occurred (16). Increased conidial germination and fungal penetration were observed at the base of root hairs and in the region near the root tip of soybean taproots, where none or very few root hairs were present. The different success rate of infection by *F. virguliforme* on the different root sites could lead to a difference in foliar symptoms expression of SDS.

The objectives of this study were to assess: 1) the importance of different infection sites to foliar symptom expression and 2) effect of root injury prior to infection on the expression of SDS foliar symptom. To accomplish our goals experiments were set up under greenhouse conditions with controlled temperature, light, and watering. Three different sites of infection were evaluated: root tip, middle, and upper root portion. Plants were evaluated for incidence and severity of foliar and root symptoms. Also, a field survey was done to compare the root symptom development in plants with symptomatic and asymptomatic foliar injury in field conditions with the greenhouse experiments.

Materials and methods

A glyphosate-resistant soybean cultivar Pioneer 92M76, known to be susceptible to sudden death syndrome, was chosen for this experiment to ease the detection of differences among treatments. Seeds were surface sterilized for 30 seconds in sodium hypochlorite solution (1% vol:vol) and incubated on sterile wet paper towel (5) at room temperature (26°C) for

germination for 3 days under 24h fluorescent light. Uniformly germinated seedlings were transferred to acrylic lids and inoculated with 10^5 macroconidia ml^{-1} spore suspension of *F. virguliforme*. Inoculation methods varied according to treatment and experiment. Different inoculated sites are shown on Figure 1.

The single-spore *F. virguliforme* isolate Mont-1 was cultured on 9-cm-diameter disposable Petri dishes containing one-third strength potato dextrose agar. Plates were incubated at room temperature under 24 h fluorescent lights. Macroconidia was harvested from 15 days old cultures under aseptic conditions by adding 10ml of distilled sterile water to each plate and using a sterilized small painting brush to release the macoconidia; the resultant conidial suspension was passed through a 3-layered sterilized cheese cloth during transfer to a 100-ml sterilized Beaker flask. A hemocytometer and an optical microscope were used to estimate the concentration of macroconidia in suspension; distilled sterile water was added to obtain a final concentration of 10^5 macroconidia ml^{-1}.

Three experiments, with slightly modified methods, were conducted to study the effect of root site of inoculation on the foliar symptoms development of SDS. **Experiment 1.** Objective of this experiment was to assess the foliar symptoms expression when the lower or upper half of tap root was inoculated. Two sets of plants had either the upper or the lower half of taproots inoculated, a 20µl inoculation loop was loaded with macroconidial suspension and used to spread it on the root site of each treatment using an up and down motion longitudinally to the taproot, this process was repeated 5 times per root so the entire surface of the site was inoculated; a third set of plants had their entire root system dipped into the macroconidial suspension as a positive control. Each treatment had 15 plants. **Experiment 2**. Objective of this experiment was to determine the effect of mechanical injury and inoculation of three root sites on foliar

symptoms expression. Three sets of plants had either the root tip, middle portion of root, or the root top inoculated with a micropipette that was used to transfer 20µl of macroconidial suspention to the root site, 2 sets of plants were either mock-inoculated with a 20µl inoculation loop and dipped into the macroconidial suspension or only dipped into the macroconidial suspension, a sixth set of plants was not inoculated. Each treatment had 32 plants. **Experiment 3**. Same treatments and objectives of experiment 2 were used, with the addition of a seventh set of plants that immediately after transplanted to cones in the green house were drench inoculated with 20ml of the macroconidial suspension. Each treatment had 40 plants.

The inoculated seedlings and uninoculated controls were transplanted into cones filled with pasteurized potting mixture of soil, sand, and peat mix at 2:1:1 ratio (vol:vol); cones were kept within buckets filled with sand leaving the upper 1cm of the cones above the sand level (12 cones per bucket); buckets were kept in a water bath (6.5 x 1 x 0.3 m) at 20°C set up within a greenhouse room; the water level was aligned with the sand level in the buckets. Water temperature was controlled by an electronic valve that opened releasing chilled water (16°C) any time that tank temperature went above 20°C, and 3 water heaters set to turn on in case water temperature went below 20°C. Two pumps at each end of the tank were used to circulate water to homogenize the tank temperature.

Plants were evaluated for SDS foliar symptoms incidence and severity. Incidence was evaluated as a qualitative binary variable, 0 when foliar symptoms were absent and 1 otherwise; severity was visually assessed as the percentage of the leaf area with typical SDS symptoms, chlorosis or necrosis. Mean severity was estimated by averaging the percentage of symptomatic leaf area of symptomatic plants only.

After the end of foliar symptom evaluation, root rot was assessed in Experiment 3 at 35 days after transplanting. Roots were washed in running tap water and evaluated for external discoloration of the taproot and lateral roots by visual determination of the percentage of surface area with typical root rot symptoms, necrotic lesions. Internal discoloration was evaluated by splitting taproots in half with a sharp blade and measuring the length of the internal discolored tissues on the first 5-cm of the root, measured from the base of stem.

The colonization of *Fusarium virguliforme* on taproots was evaluated by plating root tissue on growth media to ascertain that observed symptomatic plants were colonized by *F. virguliforme*. After splitting the taproot and measuring internal discoloration, each half taproot was dissected into 5 pieces of 1 cm and surface sterilized by submersion on solutions of 70% ethanol and 1% sodium hypochlorite for 30 seconds each, followed by rinsing twice in sterile water. After surface sterilized, root pieces were placed on a 9-cm disposable Petri dish containing modified Nash and Snyder's medium consisted of 20 g of agar, 15 g of peptone, 1 g of monopotassium phosphate (KH2 PO4), 0.5 g of magnesium sulphate (MgSO47H2O), and 1 liter of water. After autoclaving the medium for 30 minutes and cooling it to 45°C, 1 g of streptomycin, 2.4 g of pentachloronitrobenzene (75% WP), 1 g of chlortetracycline, and 0.5 g of rifampicin were added and mixed using a stirring magnetic rot and a stir plate. After 14 days, plates were examined for the presence of *F. virguliforme* colonies surrounding each root segment based on colony and macroconidia morphology. Colonization was estimated by averaging the percentage of root pieces with positive isolations in each treatment, and isolation frequency is the percentage of plants with positive isolation in each treatment.

The experiments had a complete randomized design. Treatments were considered to be the site and method of inoculation and each individual soybean plant was considered to be an

experimental unit. Incidence and isolation frequency were treated as binomial variables because each plant presented a yes/no response. Incidence was analyzed by performing a logistic regression with a Logit link function using GLIMMIX procedure in SAS 9.2 (SAS Institute, 2008); Tukey-Kramer test was applied with $\alpha=0.05$ to determine differences between treatments. ANOVA, with $\alpha=0.05$, was performed to analyze normally distributed data; colonization, foliar and root rot severity, and discoloration.

Field survey. To compare the relationship of root and foliar symptoms of greenhouse experiments to field conditions, 37 Pairs of soybean plants were collected from fields naturally infested with *Fusarium virguliforme* at the ISU-Northeast Research and Demonstration Farm in Nashua-IA, after pod fill stage, and after foliar symptoms were fully expressed in the fields during the growing season of 2011. Pairs consisted of side by side plants of which one was expressing clear SDS foliar symptoms and the other appeared to be healthy, without foliar symptoms. Visual assessments were made on the severity of foliar and root symptoms. Root rot and root internal discoloration were compared between symptomatic and asymptomatic plants. A paired *t*-test was performed to compare root symptoms between plants with and without foliar symptoms.

Results

The effect of root inoculation site on infection by *Fusarium virguliforme* and foliar symptoms was investigated in 3 green house experiments with the assessment of incidence, severity, and colonization of the plants by the fungus.

Experiment 1. Foliar symptoms were first observed 13 days after seedlings were transplanted to cones in greenhouse conditions. Incidence levels stabilized 27 days after transplant in all treatments. All treatments had similar severity levels in all six visual assessments (Fig. 2B), whereas incidence greatly varied among the treatments (Fig. 2A). When foliar symptoms were first observed the severity rate was approximated 5% on all 3 treatments. As the disease progressed over time, severity increased to over 60% in all treatments at similar rate.

When the lower portion of roots was inoculated plants had significantly higher incidence levels than when other sites were inoculated, over 90% of the plants developed SDS foliar symptoms 27 days after transplant; in contrast, when the upper portion of roots was inoculated incidence level was at 60%, and plants that had their roots submerged in inoculum suspension expressed even lower foliar symptoms with incidence level below 50%. Plants that were submerged in inoculum suspension likely came in contact with more spores than other set of plants but did not received any friction during the inoculation process, as did the plants that had part of their roots inoculated with inoculation loop

Experiment 2. In contrast to experiment 1, incidence levels in this experiment were low, below 30% (Fig. 2A), even though both experiments were conducted and controlled under same environmental conditions as far as we knew. First foliar symptoms were observed 18 days after transplant, but unlike experiment 1, the incidence on treatments that developed the disease presented a very small change over the period of the experiment. Severity, similar to experiment 1, presented great variability within treatments.

Plants not inoculated or that had only the top portion of their roots inoculated did not develop any foliar symptoms (Fig. 3A). Plants that were scraped with inoculation loop and dipped into inoculum suspension afterwards presented the highest incidence with 30% of plants

showing foliar symptoms and were statistically different from all other treatments; in comparison, when plants were submerged into inoculum without mock inoculation, incidence was approximately 10%. When plants were inoculated on either middle root portion or root tip, severity levels were approximately 15% at the end of experiment. Plants that had the middle portion of their roots inoculated and plants that were scraped and submerged into inoculum presented statistically similar values for severity, 80 and 60% respectively (Fig. 3B). These treatments also displayed incremental increase in severity values during the experiment, whereas plants that were dip inoculated presented a declined on severity in the fourth week after.

Experiment 3.Incidence levels in this experiment were even lower than levels found in experiment 2; only two treatments developed foliar symptoms, in contrast with four from experiment 2, dip inoculation and drench-inoculated plants (Fig. 4A). First symptoms were also observed 18 days after transplant similar to experiment 2. The incidence and severity of drench-inoculated plants was significant greater than that of plants dipped into inoculum suspension (Fig. 4B). Plants that were drench-inoculated had incidence levels below 25%, and dip inoculated plants were below 10%. Dip-inoculated plants also had significantly lower severity than drench-inoculated, 25 and 45% respectively, at the end of the experiment. The fact that the treatments that expressed symptoms in experiment 2 did not express symptoms in experiment 3 suggested that there is at least another unknown factor affecting foliar symptom expression.

Although the majority of plants had no foliar symptoms, virtually all of the inoculated plants showed root rot. Severity levels were similar in all but the drenched method. Treatments with no inoculation showed 5% severity, drench inoculation 55%, and all others between 10 and 18% (Fig. 5A). Once taproots were split open for the investigation of internal discoloration, it was evident that plants that were drenched inoculated had a higher incidence of internal

discoloration when compared to other treatments. Plants that were not inoculated, and plants that had their mid or top root portion inoculated had no internal discoloration. Plants that had their roots dip- inoculated or dip-inoculated + scraping had intermediate values for incidence of internal discoloration, 30 and 25% respectively (Fig. 5B). The dip-inoculation + scraping and root-tip inoculation treatments had discoloration lengths of 1.8 and 1.5 cm, respectively, whereas drench and dip inoculation had 2.9 and 2.7 cm of discoloration, respectively (Fig. 6A).

Although only drench and dip-inoculated plants had foliar symptoms of SDS, *Fusarium virguliforme* was isolated from plants of all inoculated treatments. Isolation frequency, the percentage of plants with positive isolation, was higher on the treatments with foliar symptoms; 100% on drench-inoculated plants and 85% on dip-inoculated plants. Over 75% of asymptomatic plants that were dip-inoculated after been scraped with an inoculation loop had positive isolation of *F. virguliforme*. Plants that were inoculated on the upper, middle or tip root portion had positive isolations for 50%, 30% and 28% of the plants, respectively and were significantly different from drench inoculation (Fig. 6B). The colonization level of *F. virguliforme* on plants with positive isolation was highly variable but no significant difference was detected. The drench-inoculated and dip-inoculation + scraping treatments had colonization levels of 95 and 78%, respectively, whereas the remaining treatments had frequencies between 50 and 60% (Fig. 7).

The length of internal discoloration on roots of symptomatic plants was significantly longer than the length of discoloration on asymptomatic plants (Fig. 8B), suggesting a threshold of colonization, or a critical fungal mass necessary to produce enough toxins for foliar symptoms expression. Also, the discoloration on asymptomatic plants was not negligible indicating that even though those plants had their internal tissues colonized by *F. virguliforme,* they did not

always develop foliar symptoms. Positive isolation of *F. virguliforme* and incidence of internal dicoloration were significantly greater on symptomatic plants when compared to asymptomatic plants (Fig. 8A).

Field survey. Most of the symptomatic plants collected had severity of foliar symptoms above 30%, ranging from 10 to 90% (Fig. 9). Symptomatic plants had levels of root rot and internal discoloration incidence significant higher than asymptomatic plants (Fig. 10A), similar to greenhouse results. Some plants with no foliar symptoms also had root rot and internal discoloration. Symptomatic plants also had significantly longer sections of internal discoloration than asymptomatic plants (Fig. 10B), which is in agreement with the results from our greenhouse experiments.

Discussion

Sudden death syndrome (SDS) is an economically important root rot disease on soybeans that can manifest foliar symptoms; it is caused by *Fusarium virguliforme* in North America. It is present in all major soybean production areas in North and South America. The objective of this study was to evaluate the importance of different infection sites on soybean roots that lead to foliar symptoms expression of SDS as well as the effect of mechanical injury of tap roots before and during inoculation. Three different sites of infection were evaluated, root tip, middle root portion and upper root. The mechanical injury was simulated by friction of a plastic inoculation loop on the root tissue.

Results of this study were not conclusive about the importance of infection sites and root mechanical injury in the expression of foliar symptoms of sudden death syndrome. Observations

made corroborate that root colonization by *F. virguliforme* does not necessary lead to foliar symptoms. Over 50% of asymptomatic plants had positive isolation for the SDS causal agent. Internal root discoloration length was observed to be significantly longer on symptomatic plants than on plants without symptoms, which may indicate increased colonization of tap roots of those plants. Higher penetration frequency of *F. virguliforme* near the root cap region of soybean roots, as reported by Navi and Yang (2008), may not be enough to always significantly increase expression of foliar symptoms. Over all, treatments that were wounded with inoculation loop prior to dip inoculation had a higher incidence of infection when compared to plants without scraping. These results suggest a possible role for physical injury on soybean roots prior to infection, but more studies are needed. To our knowledge, the relationship between root injury and the development of foliar symptoms of SDS is being reported for the first time. Under field conditions, root injuries can be caused by insects, in some regions, and nematodes, which are present in most soybean producing areas in US. Soybean cyst nematode has been found to be associated with the occurrence of SDS. Wounds on root surfaces can potentially disrupt a cellular mechanic protection against, in this case *F. virguliforme*. Effect of wounding on root infection has been shown in others pathosystems involving *Fusarium* species and legumes (26, 28).

Many other variables such as rainfall, planting date, soil temperature, soil organic matter content, soil fertility, plant genotypes, and different *F. virguliforme* isolates, have been found to affect occurrence of foliar symptoms for SDS, but so far a reliable prediction model has not been developed., This suggests that one or more factors that have not been identified are important for foliar symptom expression. Based on our results we suggest that tap root physical injury may be one factor affecting foliar symptom expression.

This study was limited to one soybean cultivar and one isolate of *F. virguliforme*; at least 3 additional *Fusarium* species cause SDS. Hence, there is a need for further investigation of the effect of infection site and mechanical injury on the expression of SDS foliar symptoms because different soybean genotypes present different susceptibility and different fungal isolates also may differ in aggressiveness and pathogenic behavior under different set of conditions.

Experiments done in controlled conditions have shown that plant age at the moment of infection is important for the development of foliar symptoms; plants infected early are more likely to develop foliar symptoms. In these experiments however, plants developed SDS foliar symptoms within 30 days of planting, which is different from field environments; where plants tend to develop symptoms during the reproductive stages of development (8, 9). There has been no attempt to describe the differences in infection and colonization processes in these two environments.

It is apparent that further investigation is needed to determine which factors are involved in the expression of SDS foliar symptoms in relation to root wounding. A better understanding of the disease process will enable researchers to develop better management strategies to minimize yield losses.

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Figures

Figure1. Description of root sites that were used as treatments on Experiments one, two, and three. Lower and upper regions correspond to the half of the radicle that was inoculated. Top, middle and root tip were three arbitrarily chosen equidistant sites.

Figure 2. Incidence (**A**) and severity (**B**) of sudden death syndrome (SDS) foliar symptoms on soybean plants (cv. 92M76) inoculated with 3 different methods with *Fusarium virguliforme* assessed on 13, 16, 19, 23, 27, and 31 days after transplanting (n=15). Bars show the standard deviation of the mean.

Figure 3. Incidence (**A**) and severity (**B**) of sudden death syndrome (SDS) foliar symptoms on soybean plants (cv. 92M76) inoculated with 5 different methods with *Fusarium virguliforme* assessed on 18, 21, 24, and 28 days after transplanting (n=32). Bars show the standard deviation of the mean.

Figure 4. Incidence (**A**) and severity (**B**) of sudden death syndrome (SDS) foliar symptoms on soybean plants (cv. 92M76) inoculated with 6 different methods with *Fusarium virguliforme* assessed on 18, 22, and 26 days after transplanting (n=40). Bars show the standard deviation of the mean.

Figure 5. Root rot severity (**A**) and incidence of internal discoloration (**B**) on soybean roots (cv. 92M76) inoculated with 6 different methods with *Fusarium virguliforme* assessed on 35 days after transplanting (n=40). Bars show the standard deviation of the mean.

Figure 6. Length of tap root internal discoloration (**A**) and percentage of soybean plants (cv. 92M76) colonized by *Fusarium virguliforme* (**B**) under 6 different inoculation methods with

F. *virguliforme* assessed on 35 days after transplanting (n=40). Bars show the standard deviation of the mean.

Figure 7. Colonization level of *Fusarium virguliforme* on soybean taproots with positive isolation (cv. 92M76) under 6 different inoculation methods with *Fusarium virguliforme* assessed on 35 days after transplanting (n=40). Bars show the standard deviation of the mean.

Figure 8. Colonization level of *Fusarium virguliforme* on soybean taproots with positive isolation, percentage of plants with positive isolation, and incidence of tap root internal discoloration (**A**), and internal discoloration length (**B**) on symptomatic (n=15) and asymptomatic (n=264) soybean plants (cv. 92M76) 35 days after transplanting. Bars show the standard deviation of the mean.

Figure 9. Distribution of sudden death syndrome (SDS) foliar symptom on symptomatic soybean plants surveyed in 4 fields in Northeast Research and Demonstration Farm at Iowa State University during the 2011 growing season.

Figure 10. Taproot rot severity and internal discoloration length of soybean roots from symptomatic and asymptomatic plants of soybean sudden death syndrome (SDS) collected in 4 fields at Northeast Research and Demonstration Farm, Iowa State University in Nashua Iowa during the 2011 growing season.

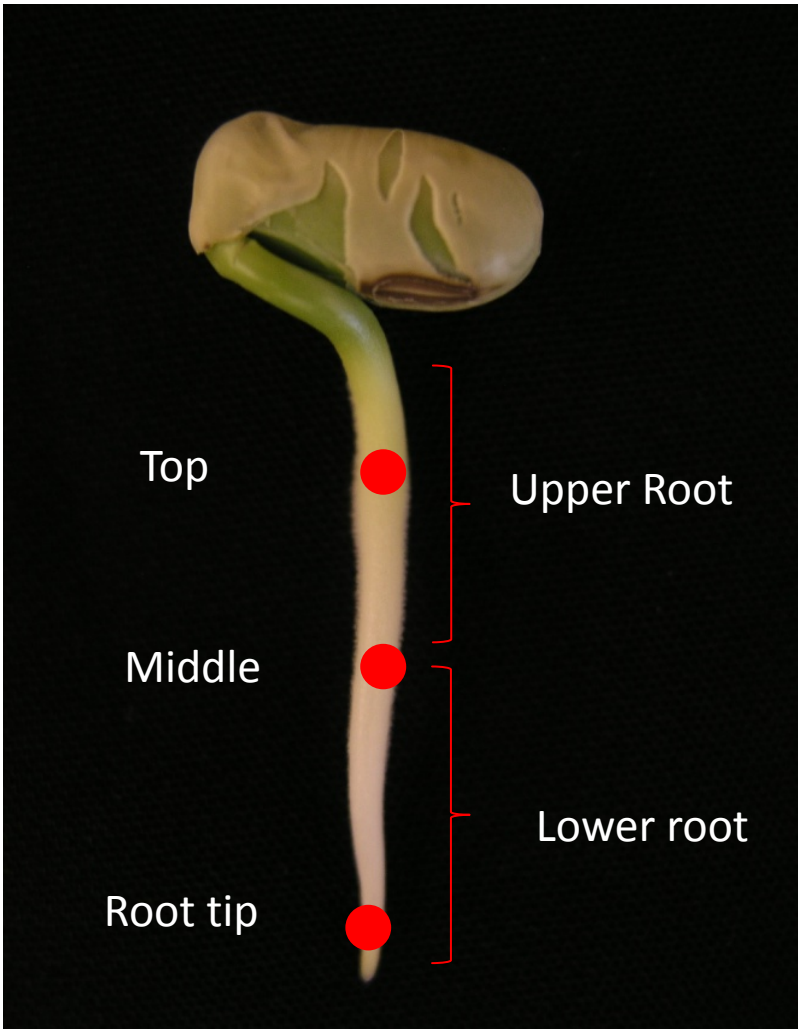
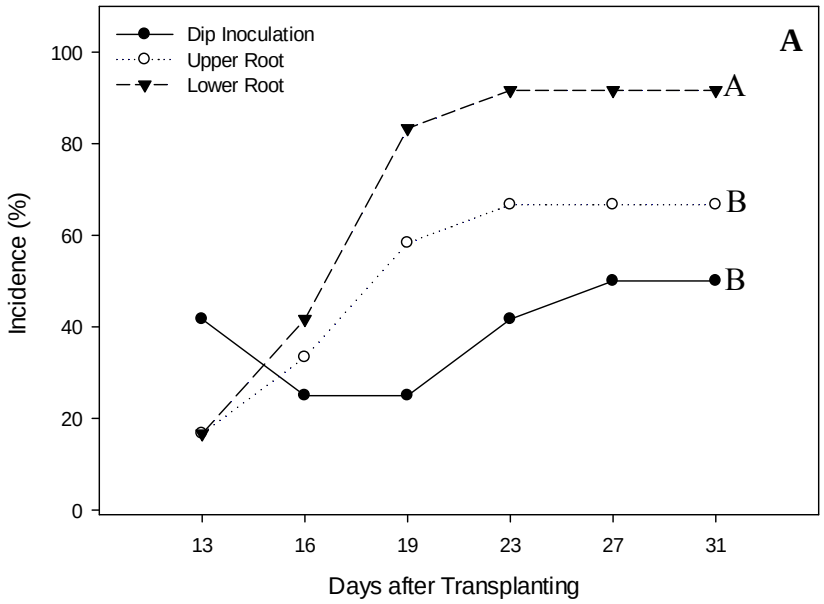


Fig. 1


A
Dip Inoculation
Upper Root
Lower Root
Incidence (%)
100
80
60
40
20
0
A
B
B
13
16
19
23
27
31
Days after Transplanting

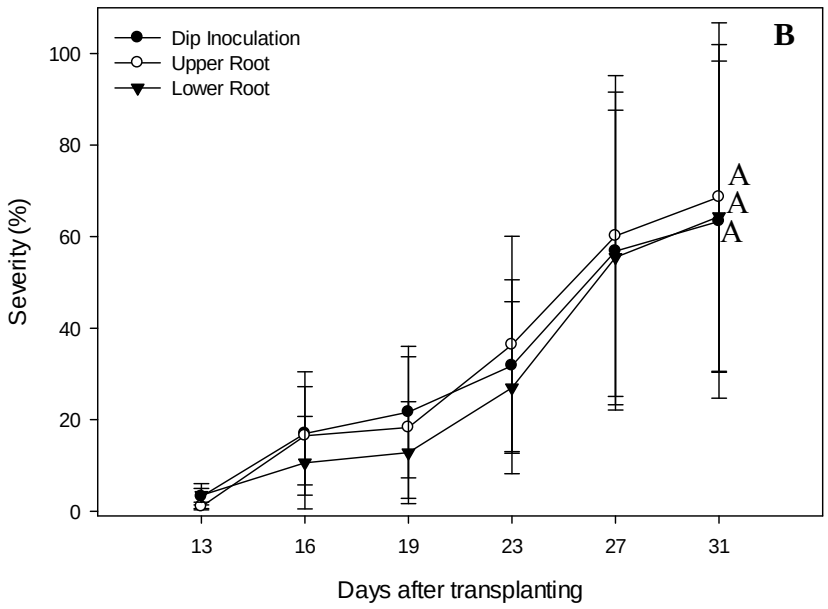
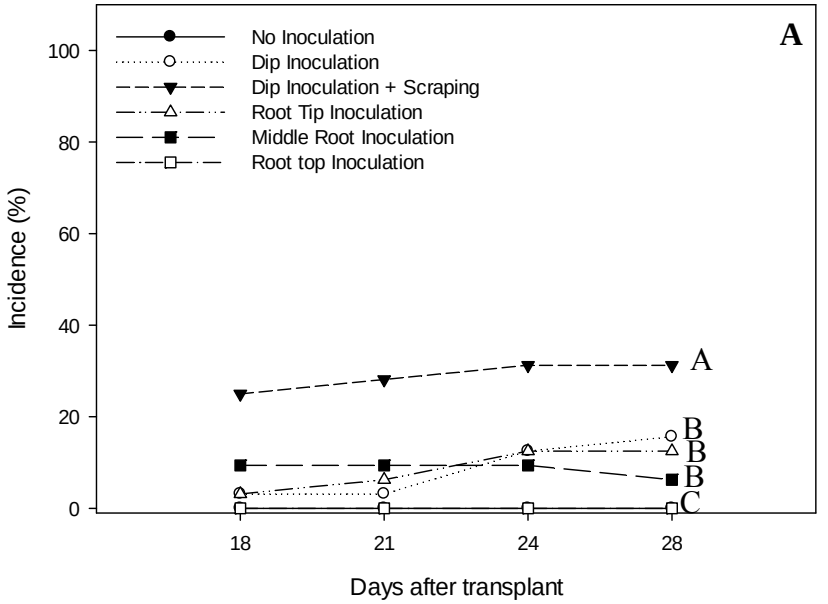

B
Dip Inoculation
Upper Root
Lower Root
Severity (%)
100
80
60
40
20
0
A
A
A
13
16
19
23
27
31
Days after transplanting

Fig. 2


A
No Inoculation
Dip Inoculation
Dip Inoculation + Scraping
Root Tip Inoculation
Middle Root Inoculation
Root top Inoculation
Incidence (%)
100
80
60
40
20
0
18
21
24
28
Days after transplant
A
B
B
B
C

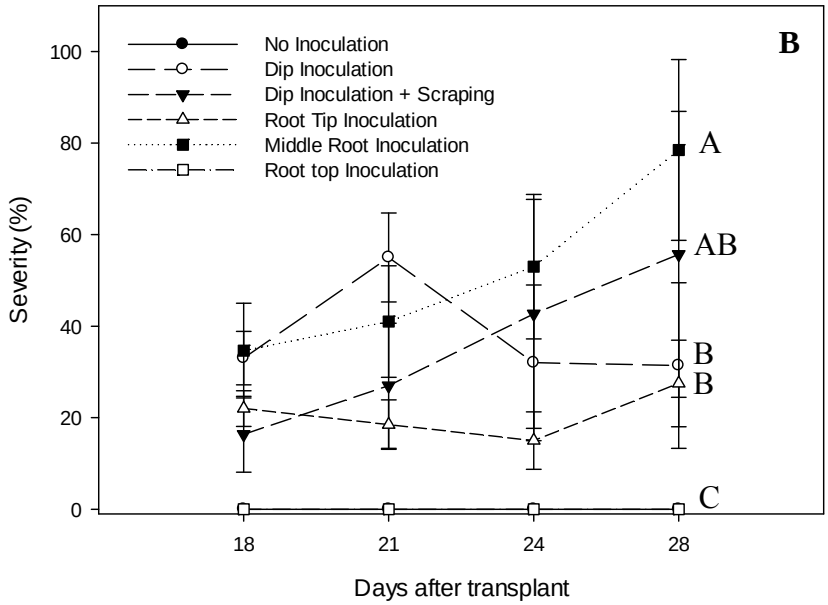
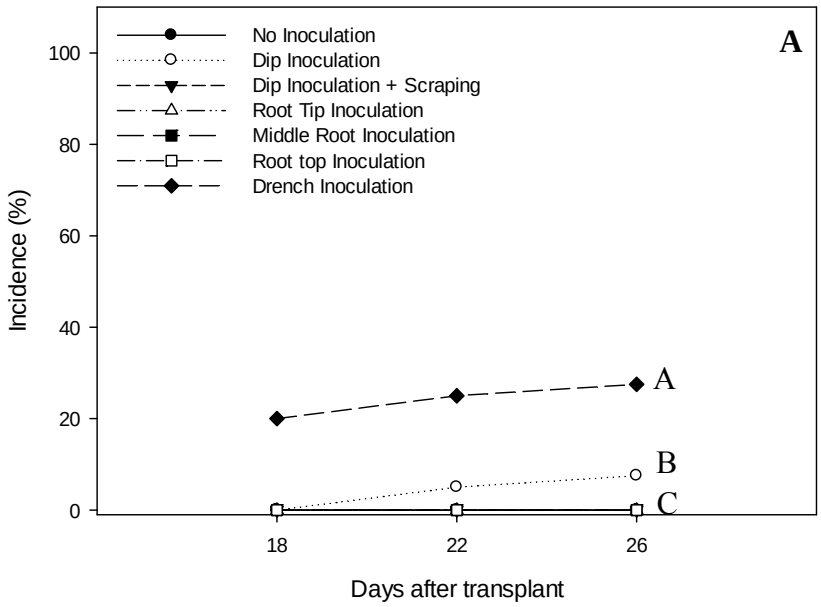

B
No Inoculation
Dip Inoculation
Dip Inoculation + Scraping
Root Tip Inoculation
Middle Root Inoculation
Root top Inoculation
Severity (%)
100
80
60
40
20
0
18
21
24
28
Days after transplant
A
AB
B
B
C

Fig. 3


A
No Inoculation
Dip Inoculation
Dip Inoculation + Scraping
Root Tip Inoculation
Middle Root Inoculation
Root top Inoculation
Drench Inoculation
Incidence (%)
100
80
60
40
20
0
A
B
C
18
22
26
Days after transplant

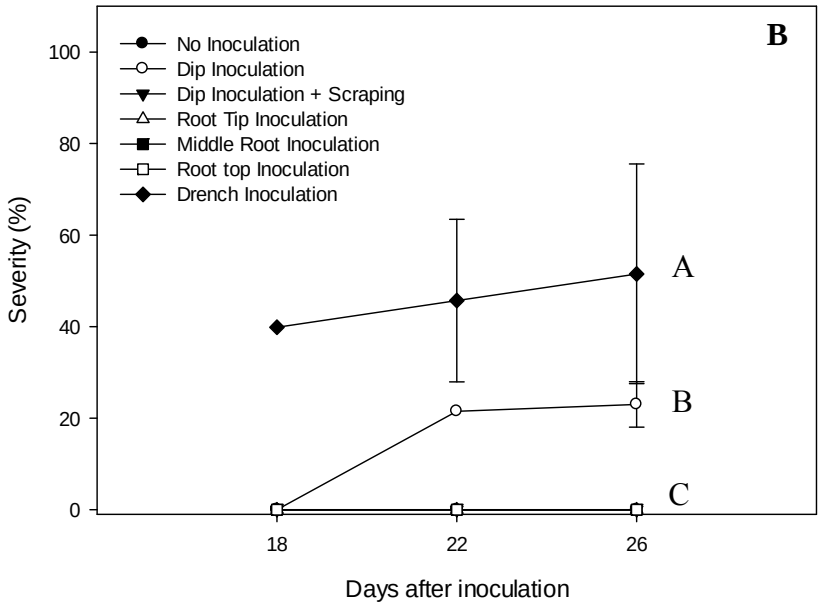
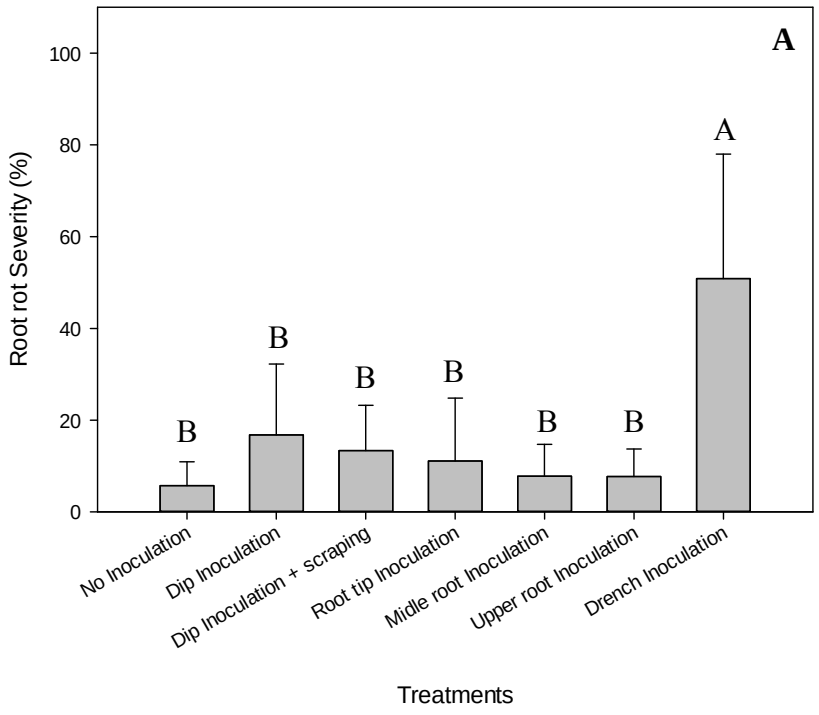

B
No Inoculation
Dip Inoculation
Dip Inoculation + Scraping
Root Tip Inoculation
Middle Root Inoculation
Root top Inoculation
Drench Inoculation
Severity (%)
100
80
60
40
20
0
A
B
C
18
22
26
Days after inoculation

Fig. 4


A
Root rot Severity (%)
100
80
60
40
20
0
B
B
B
B
B
B
A
No Inoculation
Dip Inoculation
Dip Inoculation + scraping
Root tip Inoculation
Midle root Inoculation
Upper root Inoculation
Drench Inoculation
Treatments

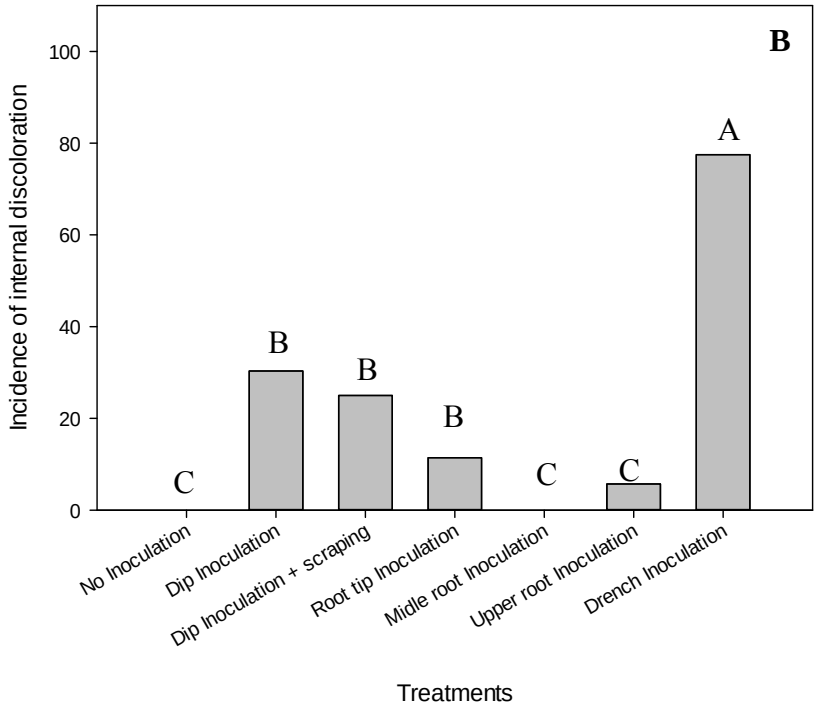
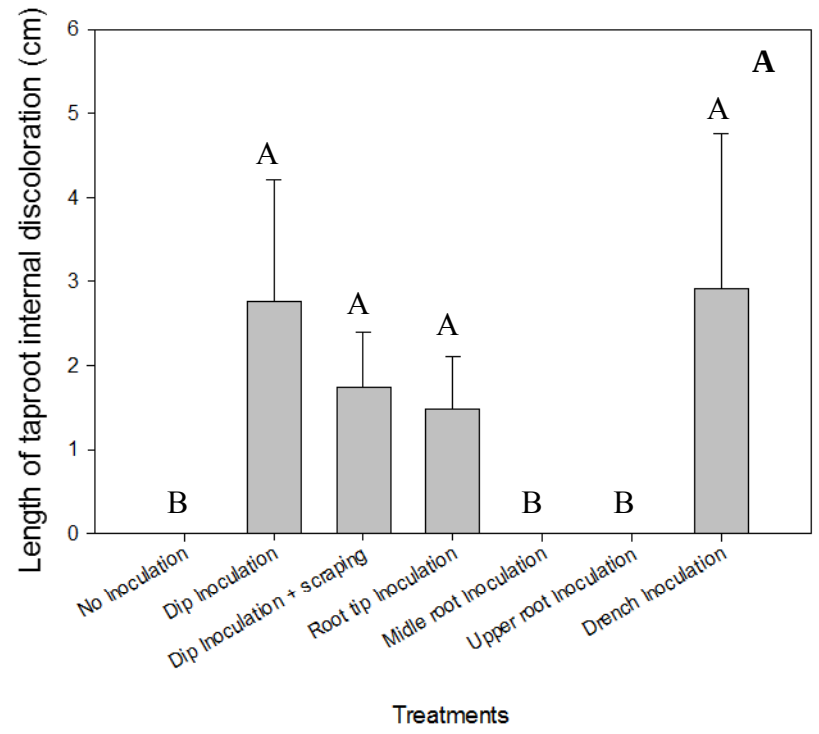

B
Incidence of internal discoloration
100
80
60
40
20
0
C
B
B
B
C
C
A
No Inoculation
Dip Inoculation
Dip Inoculation + scraping
Root tip Inoculation
Midle root Inoculation
Upper root Inoculation
Drench Inoculation
Treatments

Fig. 5


Length of taproot internal discoloration (cm)
6
5
4
3
2
1
0
A
A
A
A
B
B
B
B
No Inoculation
Dip Inoculation
Dip Inoculation + scraping
Root tip Inoculation
Midle root Inoculation
Upper root Inoculation
Drench Inoculation
Treatments

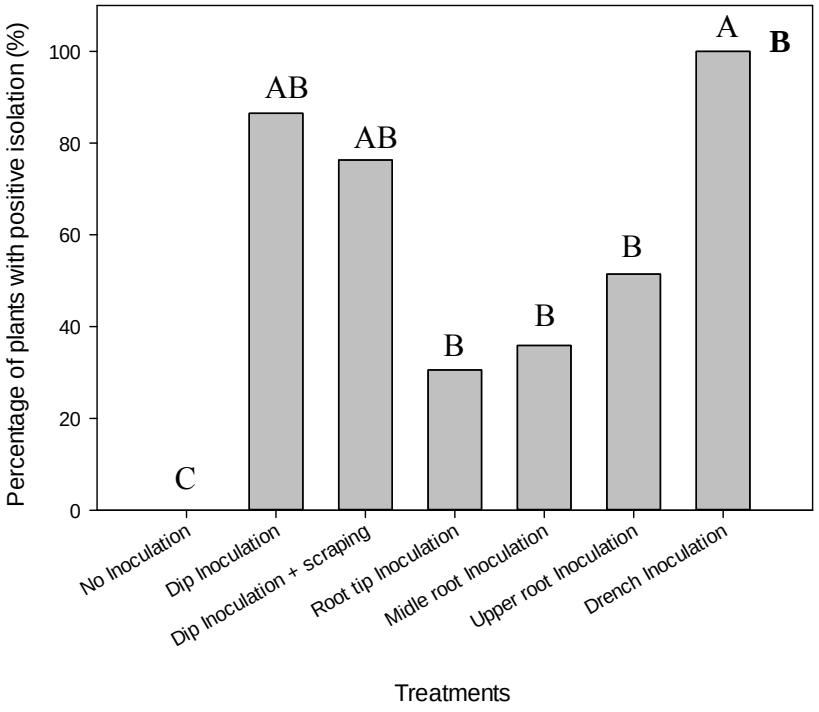

Percentage of plants with positive isolation (%)
100
80
60
40
20
0
A
B
AB
AB
B
B
B
C
No Inoculation
Dip Inoculation
Dip Inoculation + scraping
Root tip Inoculation
Midle root Inoculation
Upper root Inoculation
Drench Inoculation
Treatments

Fig. 6

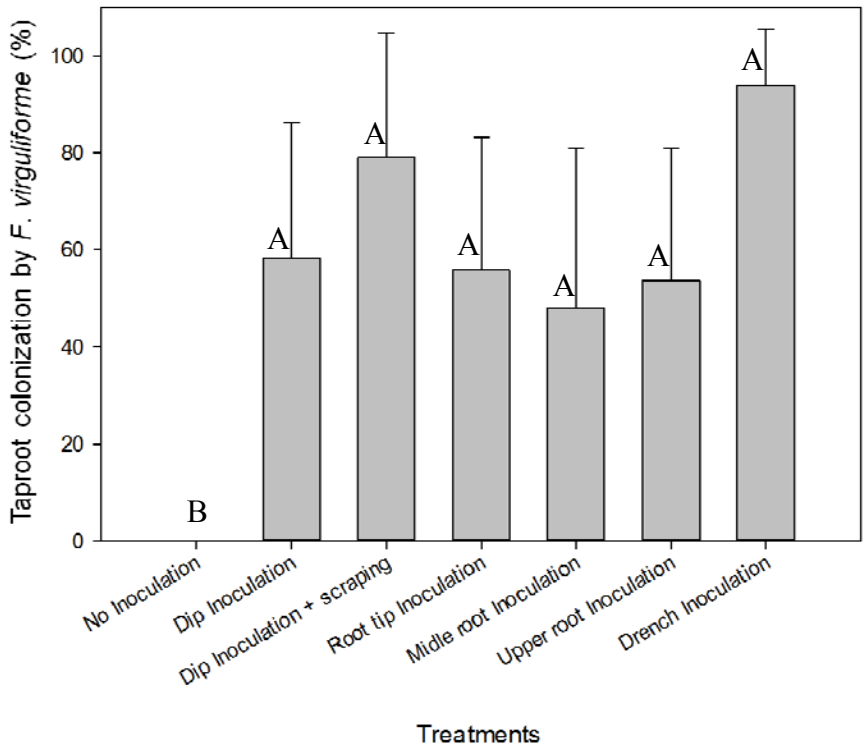

Taproot colonization by F. virguliforme (%)
100
80
60
40
20
0
A
A
A
A
A
B
No Inoculation
Dip Inoculation
Dip Inoculation + scraping
Root tip Inoculation
Midle root Inoculation
Upper root Inoculation
Drench Inoculation
Treatments

Fig. 7

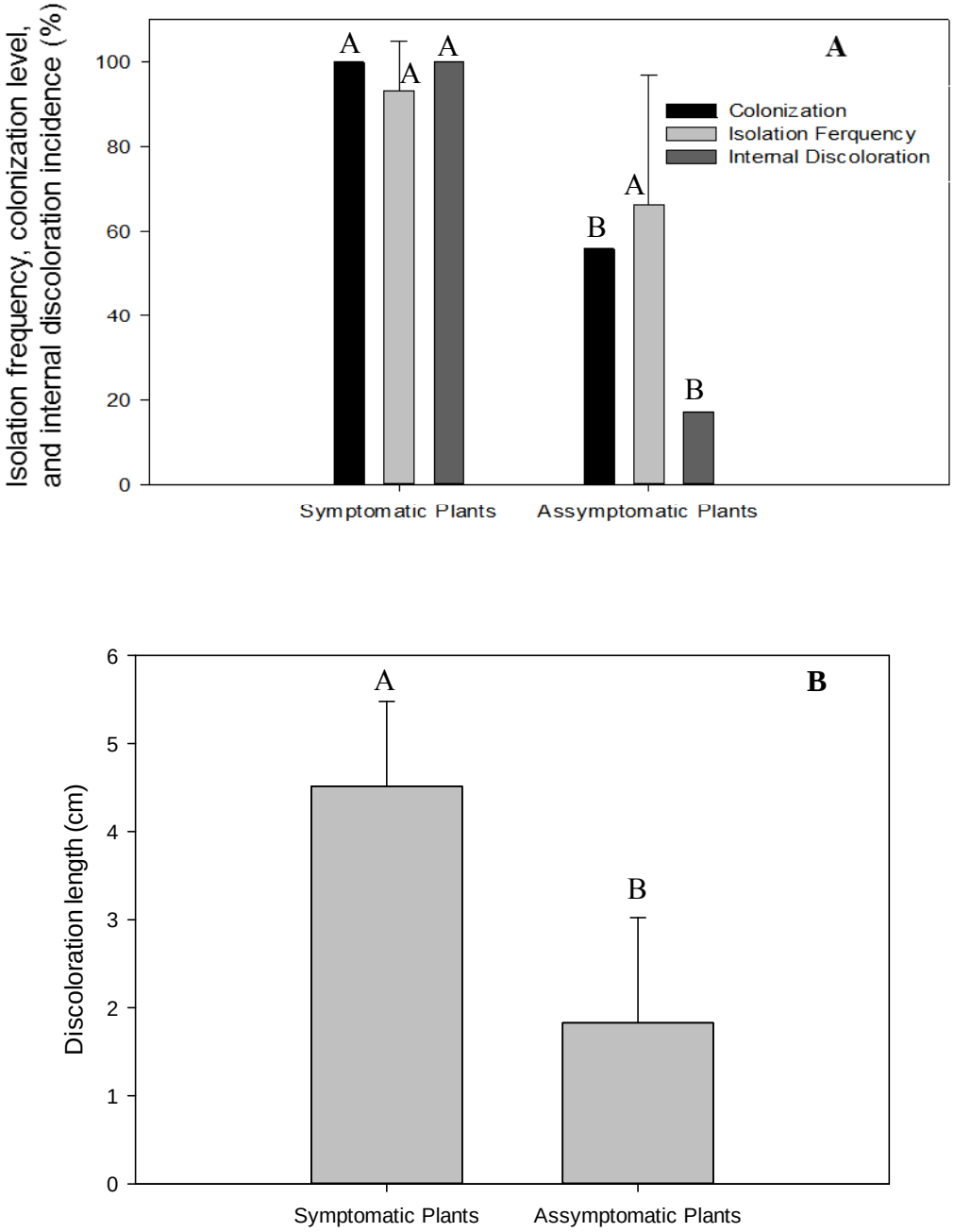

Isolation frequency, colonization level, and internal discoloration incidence (%)
100
80
60
40
20
0
A
A
A
B
A
B
A
Colonization
Isolation Ferquency
Internal Discoloration
Symptomatic Plants
Assymptomatic Plants
Discoloration length (cm)
6
5
4
3
2
1
0
A
B
B
Symptomatic Plants
Assymptomatic Plants

Fig. 8

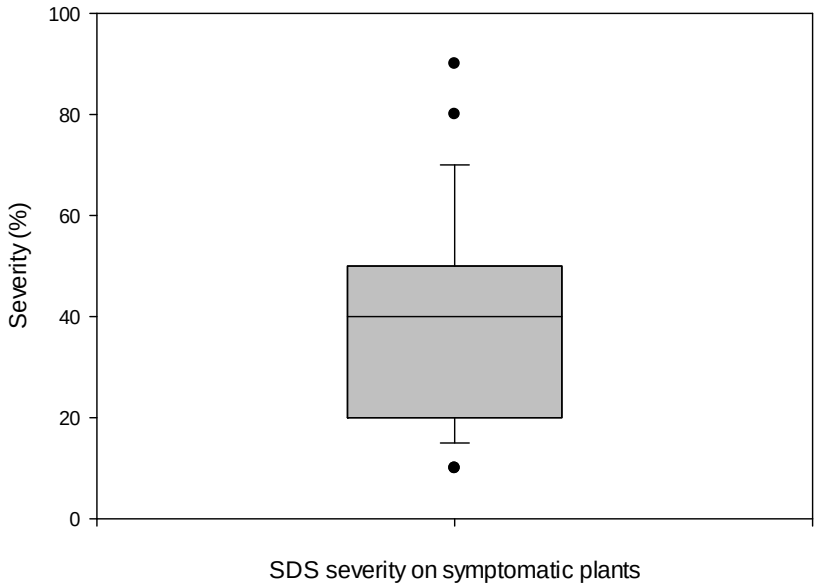

100
80
60
40
20
0
Severity (%)
SDS severity on symptomatic plants

Fig. 9

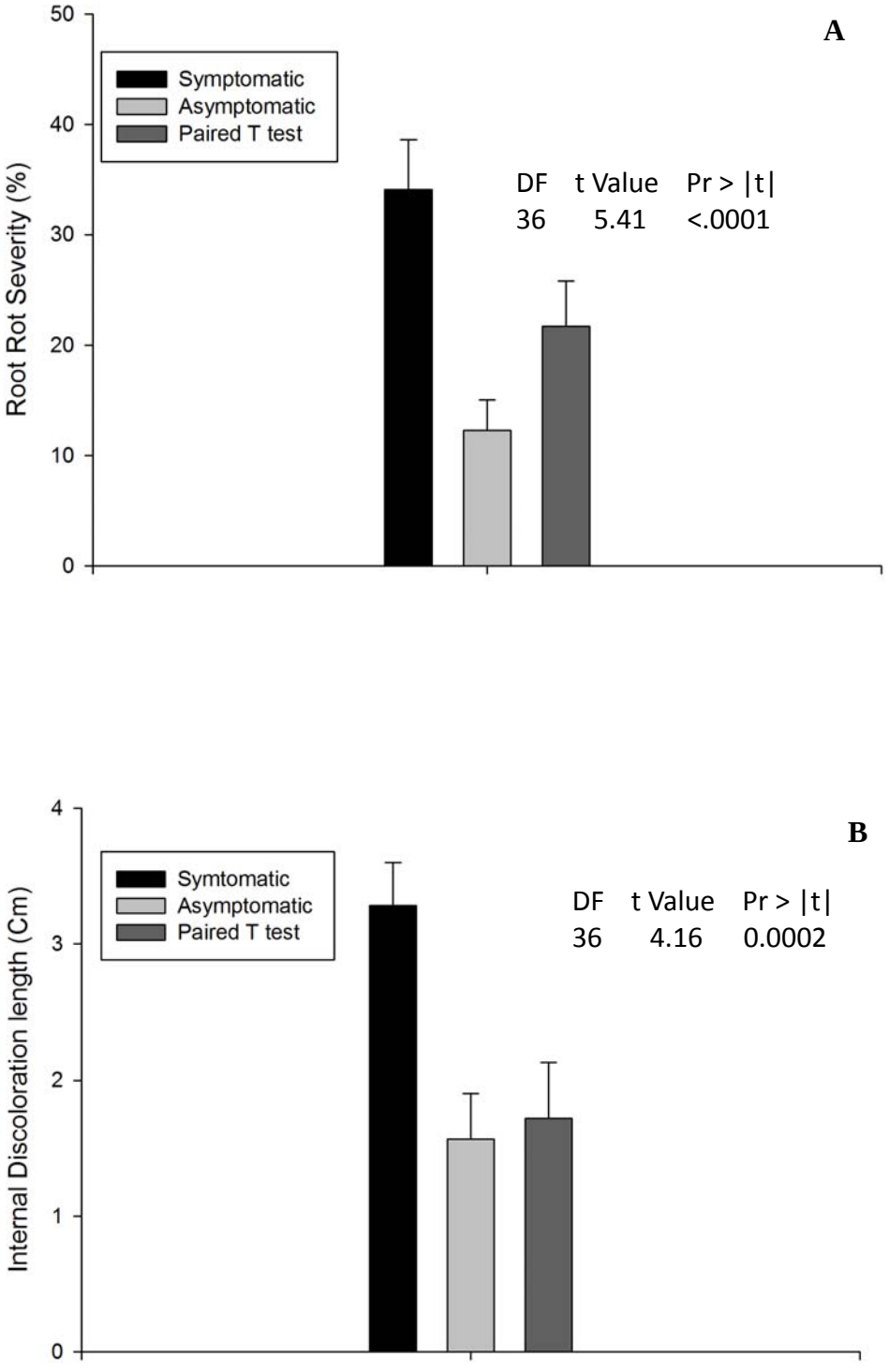

A
Root Rot Severity (%)
50
40
30
20
10
0
Symptomatic
Asymptomatic
Paired T test
DF t Value Pr > |t|
36 5.41 <.0001
B
Internal Discoloration length (Cm)
4
3
2
1
0
Symtomatic
Asymptomatic
Paired T test
DF t Value Pr > |t|
36 4.16 0.0002

Fig. 10

CHAPTER 3. EFFECT OF GLYPHOSATE ON FOLIAR SYMPTOM EXPRESSION OF SOYBEAN SUDDEN DEATH SYNDROME UNDER GREEN HOUSE CONDITIONS

Abstract

Soybean sudden death syndrome can cause severe yield losses if conditions are favorable to its development. It is endemic in all soybean production areas in the US. Glyphosate [N-(phosphonomethyl) glycine] is a post-emergent broad spectrum non-selective herbicide that is largely used in soybean production systems. Previous study (see results in Chapter 2) shows that infection does not always lead to disease in this host-pathogen interaction as the fungus can reach high level of infection of colonization and remain asymptomatic. Expression of foliar symptoms is critical to the occurrence of SDS. Research has shown that glyphosate can increase SDS disease levels on glyphosate-tolerant soybean cultivars, but due to inconsistency of results, there is the need of more research to confirm this. To evaluate the effect of glyphosate application on SDS foliar symptom development two greenhouse experiments were conducted using 4 glyphosate-tolerant soybean cultivars, P92Y51, P93Y13, P93Y13, and P93Y13, that were planted in soil inoculated with *F. virguliforme* and sprayed with 4 different application rates of glyphosate 0, 1.2, 1.6, and 2.4 L ha^{-1} of Roundup Ultra ®, plants were sprayed 2 weeks after planting. Incidence and severity of foliar symptoms were evaluated as well as plant height. Our results show that specific cultivars and rates of glyphosate can increase incidence and severity of SDS foliar symptoms.

Introduction

Sudden death syndrome (SDS) is an important disease of soybean [Glycine max (L) Merr.] (15). It is caused by *Fusarium virguliforme* in North America and different species of *Fusarium* in South America (1, 14). The disease was first observed in Arkansas in the early 70's by H. J. Walters (15), but now it is found in all major soybean growing regions, including the Midwest region of US and South America (18). The fungi colonize the roots of plants in the field causing root rot and internal discoloration of the tap root and base of the stem. Foliar symptoms, if manifested, can be observed usually after flowering. Leaves show interveinal chlorosis followed by necrosis and eventual detachment of the petiole from the stem on late stages. Yield reductions of 5-15% are often observed in fields with high incidence of the disease, if the disease onset occurs early in the season and plants develop high severity of foliar symptoms, yield losses can be up to 70% (4, 15). Leaf symptoms are thought to be caused by a pathogen-produced toxin (13, 15).

Glyphosate [N-(phosphonomethyl) glycine] is a post-emergence broad spectrum non-selective herbicide (20). After the release of glyphosate resistant soybean cultivars in 1996, there was a progressive substitution of glyphosate for many herbicides formerly used on soybeans (2). In 1994, glyphosate was used on 15% of US soybean acreage, this area increased to over 60% and 95% in 2000 and 2006, respectively (2, 3). Research has shown that the use of glyphosate can have a negative impacts on nutrient efficiency and plant diseases on diverse crops (7, 8, 16).

Research has shown that soybean cultivars that are partially resistant to SDS have higher lignin accumulation in their roots when challenged with *F. virguliforme* than more susceptible cultivars (10). Also, glyceolin, a soybean phytoalexin, has been found to inhibit in vitro growth

of *F. virguliforme* (10). Glyphosate aplication has been shown to reduce and slow down the lignin (19) and glyceolin accumulation (6) in soybean glyphosate-tolerant plants when challenged with *F. virguliforme*. Previous research has shown that soybean plants sprayed with glyphosate had higher root colonization levels of *Fusarium spp*. (11) and significantly increased the quantities of carbohydrate and amino acids secreted by their roots, which increase rhizosphere biological activity (9).

According to Sanogo *et al*., in vitro experiments have shown that glyphosate can reduce conidial germination of *F. virguliforme* as well as mycelial growth rate and the effect of glyphosate on foliar symptom expression of SDS can be influenced by fungal isolate (17). In field conditions, when glyphosate was applied 41 days after planting, the effect on disease severity and root pathogen isolation frequency was increased on plants treated with glyphosate (16). On the other hand, Njiti *et al*. reported from a one-year field study that, in field conditions, the application of glyphosate at the three leaf stage had no effect on disease parameters. Lower disease levels in the experimental areas were observed by Njiti *et al* when compared to Sanogo *et al* which could be one reason for the discrepancy.

Even though the effect of glyphosate on SDS has been researched, there is still a need for more research to resolve the contradictions. The objective of this study was to assess the effect of foliar application of glyphosate on the expression of foliar symptoms of soybean sudden death syndrome. To accomplish this goal, seeds of 4 soybean glyphosate tolerant cultivars were planted in inoculated soil under greenhouse conditions with and without foliar spray of glyphosate.

Materials and methods

To determine the effect of glyphosate on the foliar symptoms expression of soybean sudden death syndrome caused by *F. virguliforme* two greenhouse experiments were set up. Seeds of 4 glyphosate-tolerant soybean cultivars were planted in 500 ml plastic cups with a sand:soil mixture (1:2) inoculated with *F. virguliforme*, five plants were grown in each cup. Plants were sprayed with glyphosate 2 weeks after planting and evaluated for SDS symptoms to assess glyphosate effect on disease parameters.

The single-spore isolate Mont 1 was used in both trials; this isolate was chosen for presenting high aggressiveness in previous experiments. Inoculum was maintained in Petri dishes contained 1/3 strength potato dextrose agar medium at room temperature and under 24h fluorescent light. For inoculum production, white sorghum seeds were soaked in water overnight and autoclaved at 121°C and 15psi for 60 minutes, after cooling, the autoclave process was repeated again. Sterilized sorghum seeds were then transferred to sterile 1liter glass jars with lids; five 5mm plugs were transferred from two weeks old colonies of *F. virguliforme* to each jar under aseptic conditions. Infested sorghum seeds were added at the rate of 1% (vol: vol) to the soil mixture and mixed thoroughly by hand before planting. Inoculated soil was transferred to 500 ml plastic cups where 5 soybean seeds were planted equidistantly in each cup at a depth of 2 cm.

Each treatment in both experiments, had 20 cups with 5 soybean plants per cup; each cup was an experimental unit with 5 soybean plants. The Pioneer® glyphosate tolerant soybean cultivars P92Y51, P93Y13, P92M40, and P92Y20 were used in both experiments. These cultivars were chosen because they are widely used by growers and represent different tolerance

levels to SDS. On a 1-9 SDS tolerance scale, 9 being the most tolerant, the seed company categorized these cultivars as 6, 7, 4, and 6, respectively.

Experiment 1. Four soybean cultivars, P92Y51, P93Y13, P93Y13, and P93Y13, were sprayed with either 0 or 1.2 L ha^{-1} of Roundup Ultra® (480g L^{-1} of a.i.) produced my Monsanto. **Experiment 2**. The same four soybean cultivars were sprayed with 0, 1.2, 1.6, and 2.4 L ha^{-1} of Roundup Ultra ®. The herbicide was sprayed using a hand held bottle sprayer calibrated for the rate of 100 L ha^{-1}.

Plants were evaluated for incidence by counting the number of plants and the number of symptomatic plants present in each cup at the moment of evaluation. Severity was also evaluated by visually scoring the percentage of symptomatic foliar area in each plant presenting SDS symptoms. The average height of plants in each cup was assessed with a metal ruler by measuring the distance from the soil to the apex of the stem of each plant.

Incidence was treated as a binomial variable because of the low sample size whereas severity and plant height were considered normally distributed. Incidence was analyzed with the GLIMMIX procedure using Logit link function in SAS 9.2 (SAS Institute, 2008) treating cultivar and glyphosate application rate as fixed effects and environment and replication as random effects Tukey-Kramer test was applied with $\alpha=0.05$ to determine the differences between treatments.

Results

The effect of glyphosate application on soybeans was investigated in 2 green house experiments where four soybean cultivars were planted in cups holding soil inoculated with *F*.

virguliforme. Plants were sprayed 2 weeks after planting. Disease parameters, incidence and severity, were evaluated for foliar symptoms of sudden death syndrome.

Experiment 1. The application of 1.2 L ha^{-1} glyphosate did not have a significant effect across all soybean cultivars on the incidence of foliar symptoms of SDS (Fig. 1A). But it did, however, have a significant interaction with cultivars. When the effect of glyphosate was analyzed for each cultivar we observed that it was not significant for cultivar P92Y51 and P93Y13, but plants treated with glyphosate from the cultivar P92M40 had significantly higher incidence of SDS, Alternately, plants treated with glyphosate from the cultivar P92Y20 had significantly lower expressionof foliar symptoms of SDS (Fig. 2). The cultivar P92Y51 had a significantly higher incidence of SDS foliar symptoms when compared to cultivar P92Y20 and P92M40 and similar levels when compared to P93Y13 (Fig. 1B).Cultivar and glyphosate application had no detectable effect on plant height (Fig 3).

Experiment 2. Plants from the same four cultivars were submitted to 4 different application rates of glyphosate 0, 1.2, 1.6, and 2.4 L ha^{-1}. Four weeks after planting, the plants with no glyphosate application had no foliar symptoms of SDS; plants that had been treated with either1.2, 1.6, and 2.4 L ha^{-1} had similar levels of incidence (Fig. 4A) and severity (Fig. 5A). At seven weeks after planting, all plants that had been treated with glyphosate had similar levels of incidence but plants treated with 2.4 L ha^{-1} were not different from plants with no glyphosate application. At this time, plants treated with 1.2, and 1.6 L ha^{-1} were significantly different from plants with no glyphosate application. There was not a significant difference on severity 7 weeks after planting among plants that had received glyphosate application, only plants that received 1.2 L ha^{-1} of glyphosate had severity significantly higher than plants that did not received glyphosate application.

At four and seven weeks after planting, the cultivar P92M40 had significantly lower incidence of SDS foliar symptoms than the other three cultivars (Fig. 4B). Plants from the cultivars P93Y13 and P92M40 had similar levels of severity, the cultivars P92Y20 and P92Y51 also had similar levels of severity on both evaluations (Fig. 5B).

The inoculation, glyphosate application rate, and cultivar had significant effect on plant height. Inoculated plants were significantly shorter than plants uninoculated (Figure7). Plants that had received 1.6 and 2.4 L ha^{-1} had similar height and were significantly shorter than plants that received 1.2 or 0 L ha^{-1} of glyphosate (Fig. 6A). The cultivar P92Y51 was significantly taller than plants from other cultivars (Fig. 6B).

Discussion

Glyphosate was found to have no major effect across cultivars in the first experiment. In the second experiment the increase in application rate resulted in increased incidence, with the exception of the highest dose across all cultivars. Severity was not different among the treatments. Glyphosate had a significant effect on plant height across cultivars. Plants sprayed with the two highest rates of glyphosate were shorter than no glyphosate or the lowest rate.

The application of glyphosate had a significant effect on plant height on both trials. The effect of glyphosate on plant height has already been documented by other researchers (5, 12). For a weed or so after the application of glyphosate the development of light chlorosis on the upper leaves was visually noticeable when compared to treatments with no glyphosate. The assumed decrease in photoassimilation during this period could be responsible for the difference in plant height.

The effect of glyphosate on incidence of foliar symptoms of SDS was cultivar dependent, similar results were obtained by Sanogo *et al*. in greenhouse and growth chamber studies (17). For some cultivars glyphosate had no effect on increasing susceptibility to SDS; it is possible that the herbicide had a negative impact on fungal growth, negatively impacting disease progress.

The results presented in this paper are in agreement with previous experiments that have shown that in field conditions, glyphosate applied 41 days after planting had incremental effect on disease severity and root pathogen isolation frequency (16). Conversely, these results are not in agreement with Njiti *et al*. report that, in field conditions, the application of glyphosate at V3 had no effect on disease parameters.

SDS is a disease that presents many challenges and unknowns. The studies described in this paper included onlyfour soybean cultivars and were carried under controlled conditions. The response of SDS under greenhouse and field conditions is known to be different for disease parameters. As such, in future studies, it is important to evaluate the glyphosate effect on larger number of cultivars and under typical field conditions for this crop.

With the increase in usage of glyphosate worldwide and the increasing importance of SDS for soybean production, it is relevant to determine the effects that this compound could have on the development of SDS. Past studies have presented conflicting results and few studies have been done on the effect of glyphosate on SDS. Our findings show that the increased application rates resulted in increased incidence on selected cultivars of soybeans.

Our results are consistent with results reported by Sanogo et al ten years ago (16,17) that RR soybean received higher rate of glyphosate had more SDS foliar symptoms. There is need for additional studies to further explore the mechanisms of asymptomatic colonization that lead to

such results. The use of glyphosate on glyphosate tolerant soybeans greatly benefits farmers and it is an important tool for weed management, but if there are risks involved in its use they should be known and well understood. More studies should be done to better understand the mechanismsof glyphosate effects on SDS to devise management strategies to minimize its effect on the disease. A clear understanding of the effect of glyphosate on plant diseases could improve our understanding of future diseases outbreaks.

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Figures

Figure 1. Effect of glyphosate (**A**) and four soybean cultivars (B) on the incidence of soybean sudden death syndrome (SDS) foliar symptoms on experiment 1.

Figure 2. Effect of glyphosate on the incidence of soybean sudden death syndrome (SDS) foliar symptoms within four soybean cultivars on experiment 1.

Figure 3. Effect of glyphosate (B) and four soybean cultivars (**A**) on the incidence of soybean sudden death syndrome (SDS) foliar symptoms on experiment 1.

Figure 4. Effect of four glyphosate application rate (**A**) and four soybean cultivars (B) on the incidence of soybean sudden death syndrome (SDS) foliar symptoms on experiment 2.

Figure 5. Effect of four glyphosate application rate (**A**) and four soybean cultivars (B) on the severity of soybean sudden death syndrome (SDS) foliar symptoms on experiment 2.

Figure 6. Effect of four glyphosate application rate (**A**) and four soybean cultivars (B) on plant height on experiment 2.

Figure 7. Effect of *Fusarium virguliforme* inoculation on plant height on experiment 2.

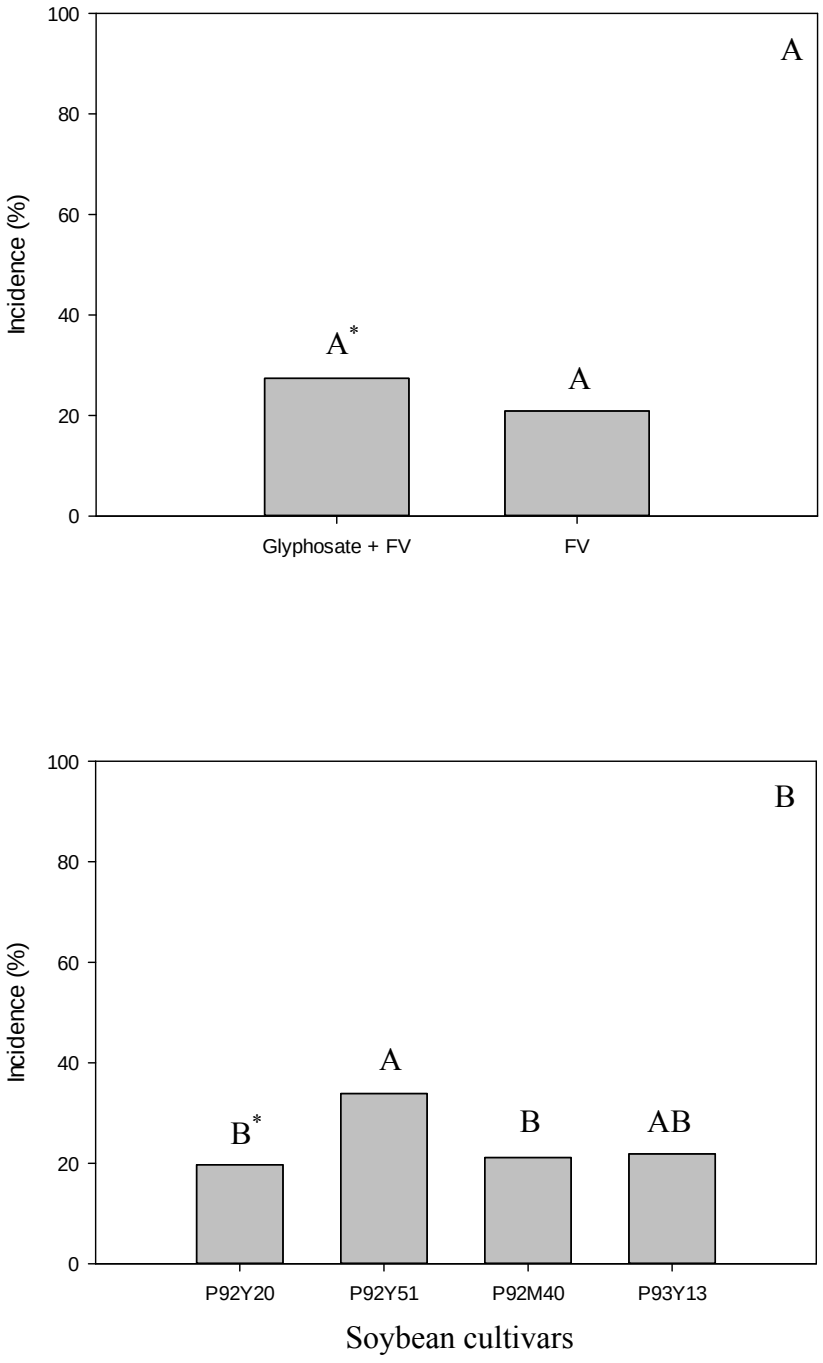


Fig. 1

* Columns with different letters are statistically different according to Tukey-Kramer test with error α=0.05.

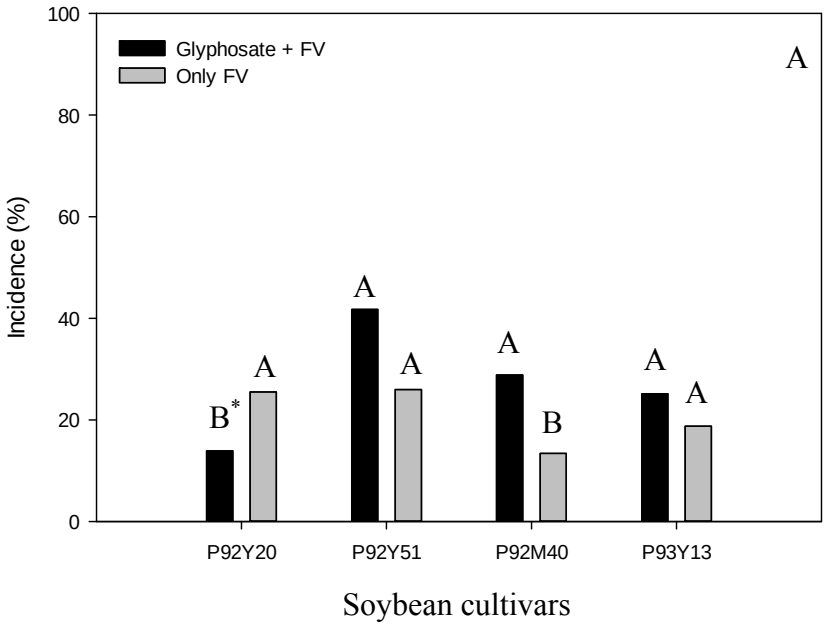


Fig. 2

* Columns with different letters are statistically different according to Tukey-Kramer test with error α=0.05.

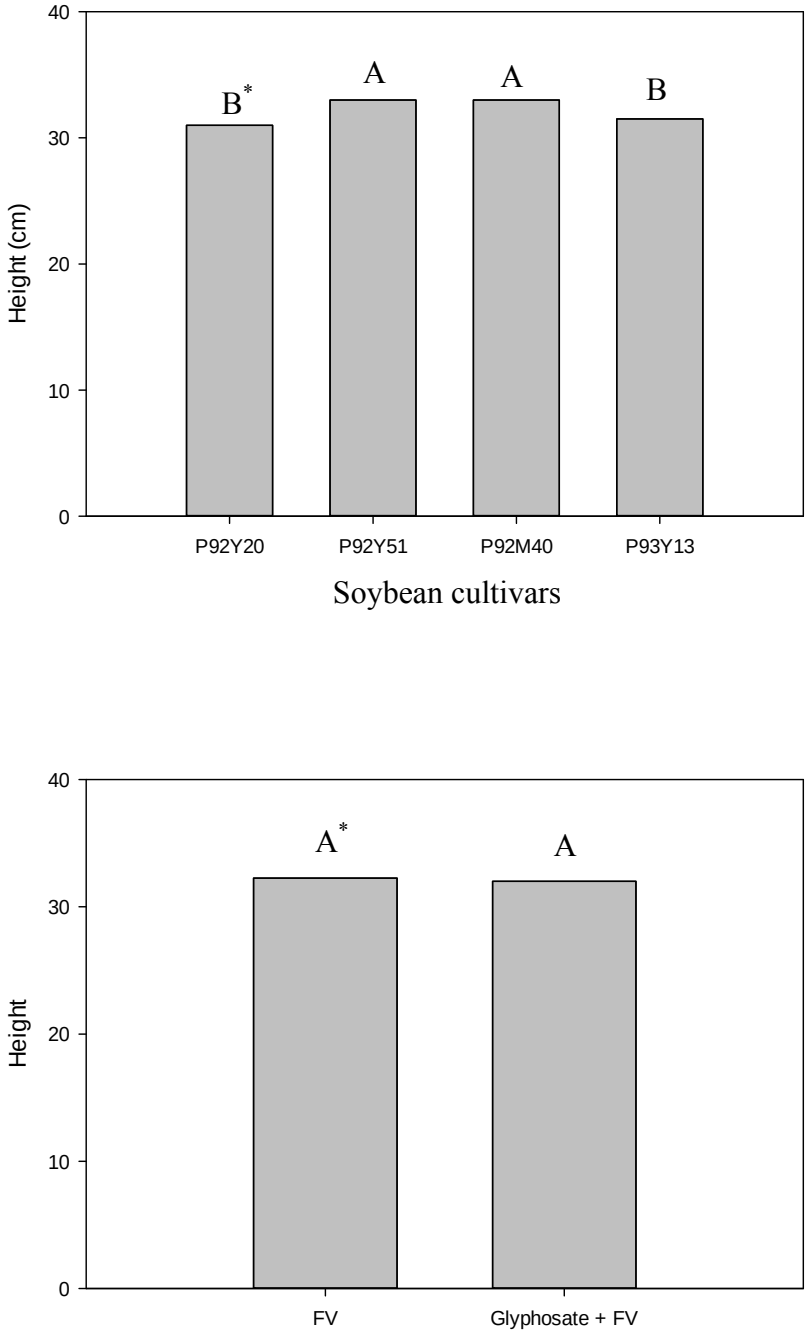


Fig. 3

* Columns with different letters are statistically different according to Tukey-Kramer test with error α=0.05. Height is in cemtermeter.

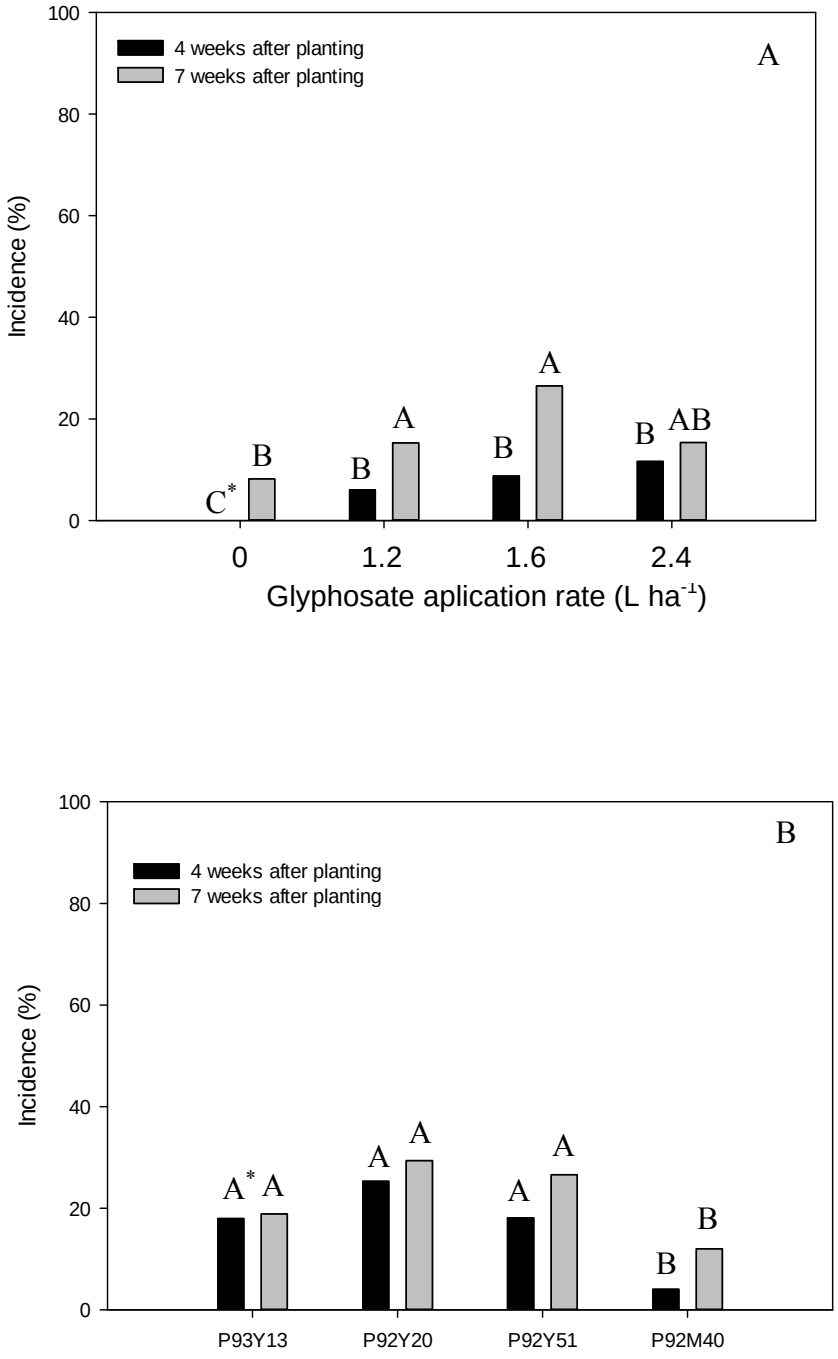


Fig. 4

* Columns of the same color with different letters are statistically different according to Tukey-Kramer test with error α=0.05.

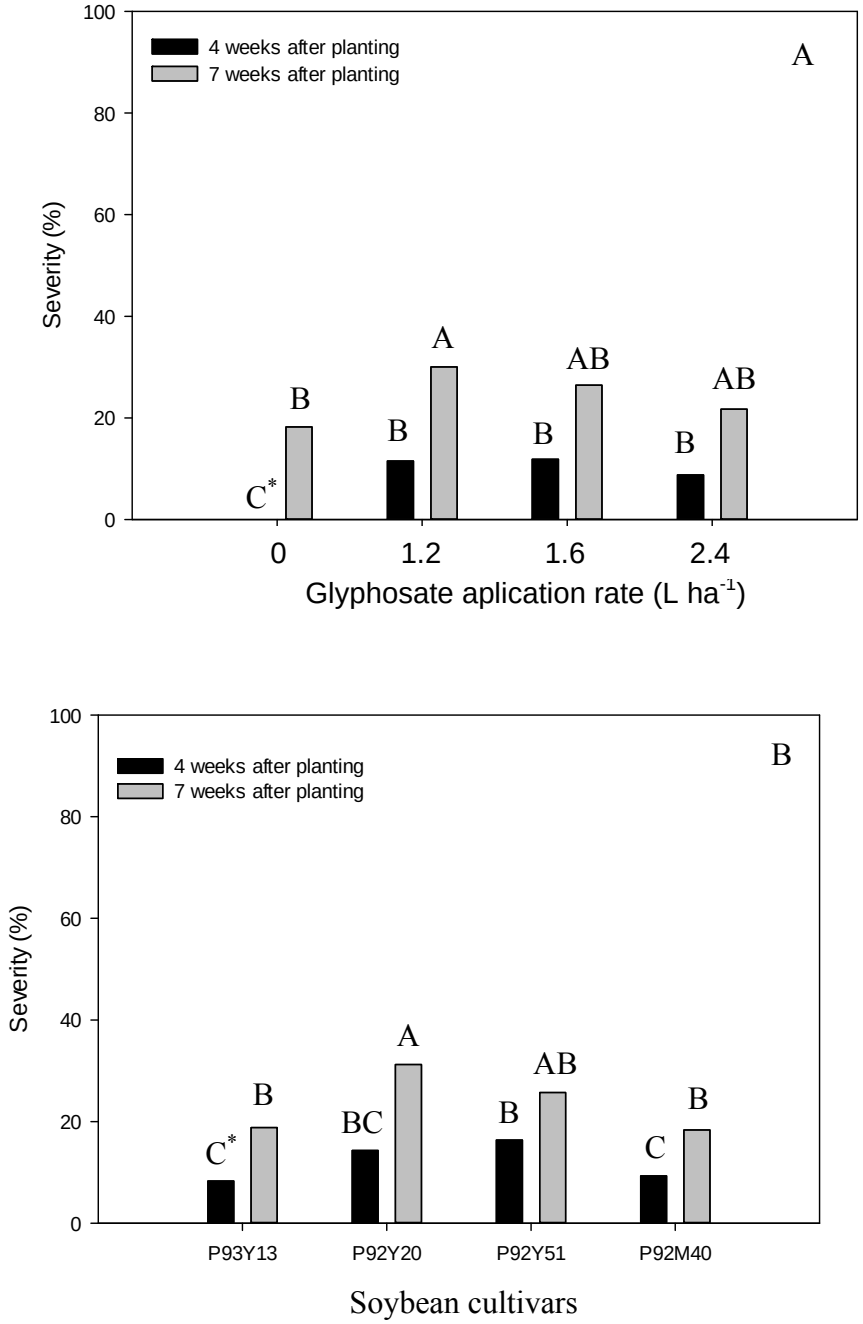
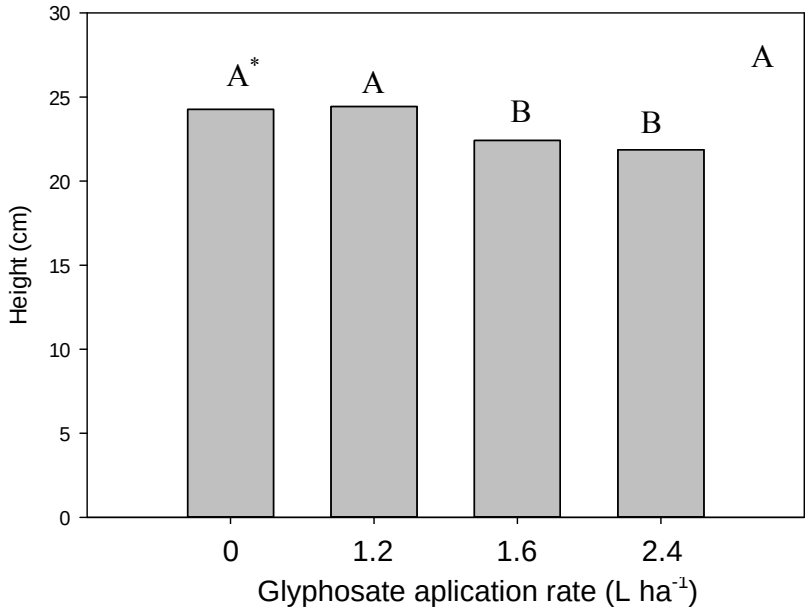


Fig. 5

* Columns of the same color with different letters are statistically different according to Tukey-Kramer test with error α=0.05.



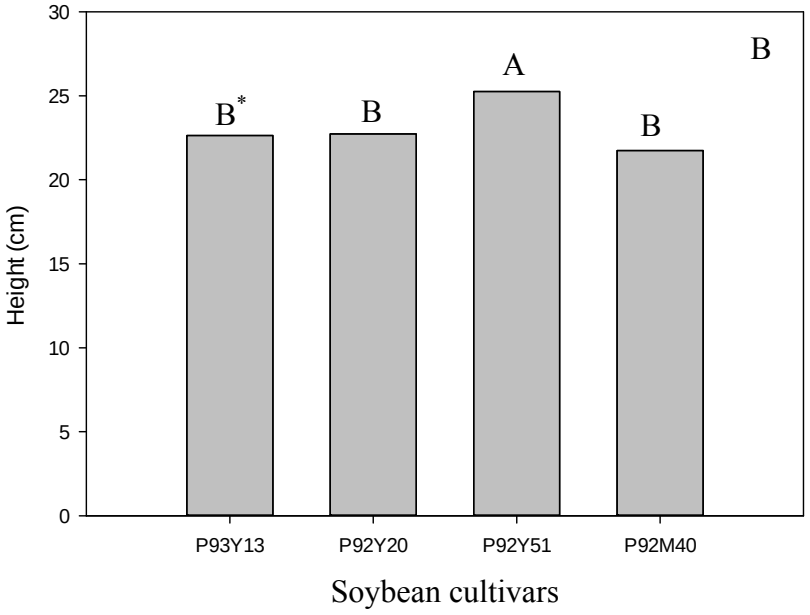


Fig. 6

*Columns with different letters are statistically different according to Tukey-Kramer test with error α=0.05.

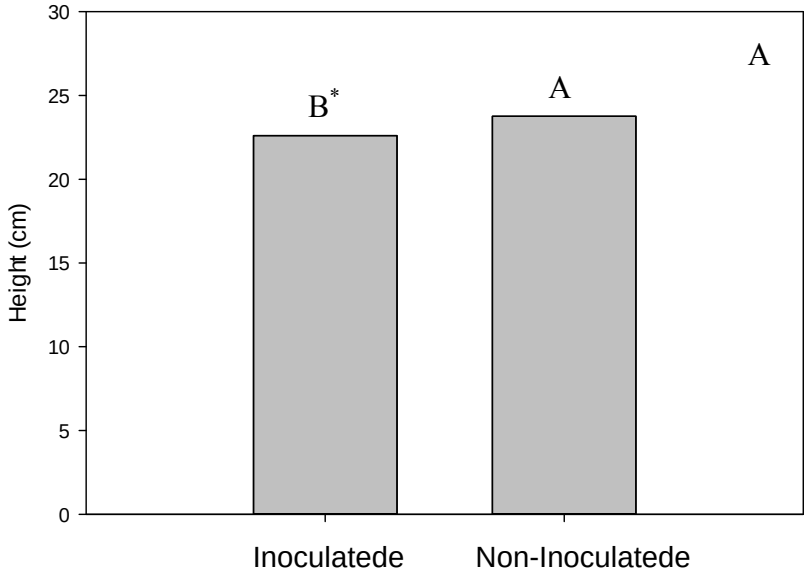


Fig. 7

*Columns with different letters are statistically different according to Tukey-Kramer test with error α=0.05.

CHAPTER 4. GENERAL CONCLUSIONS

The experiments described in this thesis studied the foliar symptom expression of sudden death syndrome of soybeans caused by *Fusarium virguliforme*. The disease parameters were evaluated in response to root infection sites and foliar glyphosate application. The data from both greenhouse study and field samples showed that *Fusarium virguliforme* can infect and colonize soybean roots, and remains asymptomatic for foliar symptoms at frequency as high as 80%. With such a high level infections in most of our experiments, we conclude that conditions are generally satisfactory for infection and level of infection is not critical to the occurrence of foliar symptom of the disease.

No significant effect of taproot inoculation site on foliar symptoms development of SDS was found. This is the first time that a study was done with the objective of evaluating inoculation sites and its consequences to foliar symptoms expression of this disease. These results could guide future research in developing more precise inoculation techniques and perhaps more effective screening methods. Also, these result show that there is more complexity to this disease than was previously known.

Glyphosate is largely used in soybean fields with the intent of controlling weeds in all growing regions. Our results show that the foliar application of this chemical increased SDS incidence on plants of some cultivars in greenhouse studies. Because the experiments were conducted in a greenhouse with under conditions of maximum favorability for disease occurrence they do not represent field conditions, therefore, results should be verified on field experiments. It is important to recognize that glyphosate is an exceptional tool for farmers to

control weeds in soybeans and has many benefits for soybean production. But the fact remains that according to our findings it can increase SDS incidence.

The difference in the infection and colonization processes that lead to expression of foliar symptoms has not been an objective in many studies. Gongora-canul (2011), in greenhouse experiments, presented the idea that infection timing is important to aerial symptom development. Navi and Yang (2008) showed that conidia germination and fungal penetration are greater at root hair base and that xylem colonization is essential for foliar symptoms expression in the greenhouse. Most studies on SDS evaluate the effect of a single variable on foliar symptoms parameters; there is little information on the effect of those variables in the infection and colonization process. Very little of the mechanisms surrounding these processes is known. New technologies such as the development of transformed strains of *F. virguliforme* tagged with green fluorescent protein and the development of an antibody for the toxin identified to induce SDS foliar symptoms could aid in more detailed investigations of colonization and infection processes as well as the production of toxin under different environments and in response to diverse variables. The results of such investigations could offer important insights on the events that lead to yield losses.

Since SDS was first documented in early 70's it has gradually spread throughout all soybean production regions North and South America. In South America, reports of yield losses due to this disease also have increased since it was first reported in that region. And the fact that there is no effective management for this disease should prompt researchers to pursue the mechanisms related to foliar symptoms expression because the disease will not happen if there was only asymptomatic infection or colonization in this pathosystem.