RESISTANCE OF SOYBEAN [*Glycine max* (L.) Merr.] TO *Fusarium solani* f. sp. *glycines*, CAUSAL AGENT OF SUDDEN DEATH SYNDROME

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ABSTRACT

Sudden death syndrome (SDS) caused by the soilborne fungus *Fusarium solani* f. sp. g*lycines* (FSG) is a major disease in soybean [*Glycine max* (L.) Merr.]. Selection for SDS resistance in the field is difficult because of the impact of the environment on disease development. The objective of my first study was to evaluate the effect of field inoculation methods, soil compaction, and irrigation timing on the occurrence of SDS symptoms. Six treatments which included FSG infested grain of white sorghum [*Sorghum bicolor* (L.) Moench], popcorn (*Zea mays everta*) or oat (*Avena sativa* L.) were planted in the furrow with the soybean seed, broadcasted and incorporated into the soil prior to planting or placed below the soybean seed just prior to planting. Three experiments were also conducted to evaluate the effect of compaction and irrigation on SDS symptom occurrence. Irrigation treatments that included water application at V3, V7, R3, R4 and/or R5 growth stages were applied. In all experiments disease incidence (DI) and disease severity (DS) ratings were taken to evaluate foliar SDS symptom and a disease index (DX) was determined. The inoculation methods that produced the most severe foliar symptom included placing infested sorghum below the seed prior to planting (DX=36.1) and planting infested popcorn in the furrow with the soybean seed (DX=28.7). No significant effects of soil compaction on SDS foliar symptom development were observed. The irrigation treatments during mid to late reproductive growth stages resulted in the greatest increases in SDS foliar symptom development.

Evaluation of a great number of lines for SDS resistance in the field is time consuming and expensive. The objective of the second study was to evaluate two SDS greenhouse screening methods and determine which best correlates with field resistance of soybean genotypes. Three sets of genotypes were previously evaluated for field reaction to SDS. All three sets were evaluated with the greenhouse cone method and two sets were evaluated with the greenhouse

tray method. For both methods plants were inoculated with FSG infested white sorghum grain and foliar symptom severity (DS) was rated 21 days after emergence. The correlations between the SDS ratings of genotypes in the field and in the greenhouse with cones ranged from 0.58 ($p<0.001$) to 0.69 ($p<0.001$). The correlations of SDS ratings of genotypes between field and greenhouse tray ratings was 0.54 ($p<0.001$) for Set 1 and 0.39^{ns} for Set 2. I concluded that the cone method has successfully predicted field results for all three sets evaluated, and hence can be used for predicting field SDS resistance reactions of genotypes.

The objective of my third study was to detect QTL conferring SDS resistance in two populations of recombinant inbred lines (RILs) populations. These populations were from the crosses PI 567374 x 'Omaha' and 'Ina' x LN91-1695. These two populations were evaluated for SDS seedling resistance in the greenhouse and the Ina x LN91-1695 population also was evaluated for SDS resistance in the field. Six chromosomal regions were significantly associated with SDS resistance in the PI 567374 x Omaha population. One significant region located on LG D2 had been previously mapped in another population. This region was identified with Satt311 ($P = 0.0032$, $R^2 = 12\%$). A second significant region was identified with Sat_299 ($P = 0.0009$, $R^2 = 12\%$) on LG I. For both regions, the beneficial alleles were derived from PI 567374. Five chromosomal regions were associated with SDS resistance in the Ina x LN91-1695 population. Two regions previously found to be associated with SDS resistance in the cultivar Essex were identified by Satt270 ($P = 0.0028$, $R^2 = 12.7\%$) on LG I and by Satt371 ($P= 0.02$ $R^2 = 12\%$) on LG C2. The beneficial alleles for both regions were from the resistant parent Ina. A second region on LG I was mapped by Sct_189 ($P= 0.0139$, $R^2 = 9.8\%$) and the beneficial allele was from the susceptible parent LN91-1695. Two additional regions were mapped onto LG A1 by Satt684 ($P= 0.02$ $R^2 = 8.5\%$) and onto LG E by Satt268 ($P = 0.01$, $R^2 = 9.8\%$) and their

beneficial alleles were from Ina. Three out of five QTL mapped with the field data were also mapped with the greenhouse data.

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INTRODUCTION

Sudden death syndrome (SDS) is a mid to late season fungal disease caused by *Fusarium solani* f. sp. g*lycines* (FSG) that can cause severe yield loss to soybean [*Glycine max* (L.) Merr.] (Gibson et al., 1994). First documented in the US in 1971 in Arkansas, the disease now occurs in most soybean producing states (Rupe et al., 1989). In Illinois, SDS was first identified in 1986 (Roy et al., 1997) and now has been confirmed throughout the state. In 2001, the losses caused by SDS in Illinois were estimated to be $150 million (Pederson, W., personal communication)

Fusarium solani f. sp. g*lycines* is characterized by slow growth in culture, and the production of abundant macroconidia in a slimy pionnotal blue green to blue purple mass. The mycelium is grayish white and may be visible on the outer edge of the culture. The macroconidia (3.8-5.0 x 34-66 :m) have three to five septa and are produced on short, simple to branched conidiophores on monophiales. Microconidia are rare. Clamydospores are globose to subglobose and terminal or intercalary in hyphae and may form in or adjacent to macroconidia. The optimum temperature for growth on agar media is 25° C (Hartman et al., 1999). Molecular studies of isolates from different states indicate that FSG is genetically homogeneous and distinct from other, non-SDS causing *F. solani* isolates (Hartman et al., 1999; Arruda et al., 2005). Recently Aoki et al. (2004) reported that SDS of soybean in North and South America is caused by two distinct species, namely *Fusarium virguliforme* in North America, and *Fusarium tucumanie* in South America. In this manuscript the causal agent of SDS will be referred to as *Fusarium solani* f. sp. g*lycines* (FSG).

Even though variation of the ability of different isolates of FSG to cause disease has been reported (Huang and Hartman, 1998; Killebrew et al., 1988; Li et al., 1999), there is no evidence of races of this pathogen (Roy et al., 1997). Recently, Mueller (2001) screened moderately

resistant plant introductions (PIs) against several FSG isolates and found different levels of disease, but no isolate x genotype interaction.

The fungus is soilborne and infects plants through the roots. The infection probably occurs soon after seedling emergence and continues throughout the life of the plant. The pathogen is restricted to the taproot and lower stem (Rupe, 1989; Roy et al., 1989), resulting in a reduction of both root mass and number of viable root nodules. The above ground symptoms include interveinal chlorosis and necrosis of leaves, premature defoliation, and pod abortion (Hartman et al., 1997). The foliar symptoms develop rapidly usually during the reproductive stage of soybean growth (Gibson et al., 1994)**.** The first leaf symptoms are circular to irregularly shaped, scattered, pale green to chlorotic interveinal spots. These spots are a few millimeters or more in diameter and produce a mottled appearance. The spots may enlarge and become necrotic, or they may coalesce and form elongated regions of interveinal chlorosis. Eventually, part or all of the chlorotic tissue becomes necrotic and green tissue remains only near the major leaf veins. Some severely diseased leaflets may drop off, leaving the still-attached petioles bare. Complete defoliation may occur when disease is severe (Roy et al., 1997). These leaf symptoms are proposed to be caused by toxins produced by the fungus in the roots and translocated to foliage (Li et al., 2000).

According to Hartman et al. (1999), foliar symptoms of SDS are similar to those of brown stem rot caused by *Phialophora gregata*, red crown rot caused by *Calonectria pyrochroa* and stem canker caused by *Diaphorthe phaseolorum* var. *meredionales*. Brown stem rot (BSR) is distinguished from SDS because BSR infected plants have a distinct discoloration of the pith, which is absent with SDS infection. Red crown rot produces red perithecia at the soil line, which

are absent in plants with SDS, and plants affected by stem canker have cankers on the lower stems that are not found on plants with SDS.

Reductions in yield ranging from slight to nearly 100% (Hartman et al., 1999) result from infection and symptom development. Gibson et al. (1994) found that yield reductions are imperfectly correlated ($r < 0.60$) with leaf symptom development. According to Nijti et al. (1997), root rotting caused by the fungus can be significant in the field but the degree of association with leaf symptom development or yield loss has not yet been adequately determined. More recently, Luo et al. (2000) reported a linear decrease in soybean yield as SDS leaf symptoms increase. Sudden death syndrome affects yield by decreasing seed size and seed number. Reduced seed number results from flower, seed and pod abortion and reduced seed size is probably due to the combination of reduced plant development, premature plant death and less capacity of the plant to produce seed dry matter (Roy et al., 1997).

The association of high soil moisture with the occurrence and severity of SDS is a common field observation (Roy et al., 1997; Ringler, 1995). Melgar et al. (1994) reported that the incidence and severity of SDS were greater on irrigated than on non-irrigated plants. Scherm and Yang (1996) reported a positive association between soil moisture and SDS severity, showing that soil moisture plays an important role in the relationship between SDS severity and yield loss. In the greenhouse, Roy et al. (1989) also found a positive relationship between soil moisture and SDS incidence.

Cool temperatures also have been positively associated with SDS expression (Nijti et. al., 1998). The results of Scherm and Yang (1996) indicate that high soil moisture and low temperature during the early part of the growing season, but warmer temperatures during

soybean reproduction, are optimal for SDS symptom expression. In the greenhouse, foliar symptoms are most severe at 20-25° C (Hartman et al., 1999).

Other factors associated with increased levels of SDS are high soil fertility (Roy et al., 1997) and soil compaction (Hartman et al., 1999). Because SDS is favored by high soil moisture, deep tillage of poorly drained fields to break up compaction is one practice that can reduce SDS occurrence. Vick et al. (2003) reported that subsoiling of compacted fields dramatically reduced foliar symptoms of SDS and this practice can be used to reduce severity of foliar symptoms where SDS occurs and soil compaction exists. Additional practices that can reduce losses from SDS include delaying planting and planting early cultivars (Hershman, 1996). Even though some authors (Von Quale et al., 1989) found less severe SDS symptoms on a corn-soybean-wheat system than on continuous soybean, crop rotation appears to have little or no impact on SDS (Hirrel, 1987).

The association between soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) infestation and SDS occurrence has been studied. Hartman et al. (1995), found no association between SDS occurrence and the presence of SCN, whereas some studies (Melgar et al., 1994; Scherm et al., 1998) report that the presence of SCN favors the development of SDS.

There is no feasible chemical control for SDS. Although studies reported that the presence of some fungi (Hershman, 1996) and bacteria (Catellan et al., 2002) can reduce SDS incidence in a greenhouse, there is currently no practical biological field control for SDS.

The use of resistant cultivars is the most effective method for controlling SDS. Some cultivars and lines with good levels of resistance have been identified (Hartman et al., 1997; Hartwig et al., 1996; Schmidt et al., 1999; Mueller, 2001). However, Mueller (2001) reported that among 1670 cultivars evaluated, only 2% were classified as moderately resistant,

emphasizing then the need for more resistance in cultivars. In the greenhouse, monogenic resistance to leaf scorch has been reported in the cultivar Ripley (Stephens et al., 1993). Under field conditions, resistance to SDS was described as polygenic (Hnetkovsky et al., 1996; Chang et al., 1996) and conditioned by a minimum of five loci in cultivar Forrest (Njiti et al., 1996; Meksen et al., 1999). Resistance to SDS appears to be partial (Nijti et al., 1996; Igbal et al., 2001) since all soybean genotypes tested have shown some SDS symptoms under severe disease pressure. In addition, field resistance was shown to be conferred by genes conferring leaf scorch resistance (Gibson et al., 1994) and root resistance (Njiti et al., 1997; Prabhu et al., 1999)

Field studies to measure the reaction of cultivars to SDS usually have been carried out in fields with histories of SDS symptoms during previous years (Nijti et al., 1996). However, selection for SDS resistance in the field is difficult because of the sensitivity of symptom development to environmental factors (Gibson et al., 1994; Nijti et al., 1996). Due to the quantitative nature of the trait and the interactions between resistance loci and the environment, effective selection for field resistance requires multiple environments (Nijti et al., 2001). In addition, the occurrence of SDS in a field is unpredictable, and the disease is often not present when SDS experiments are conducted (Diers, personal communication)**.** Artificial inoculation methods have been used in the field, however, the efficiency of these methods is not clear. Stephens et al. (1993) used microplots to evaluate reactions of 12 soybean cultivars to SDS in soil both naturally infested and inoculated with FSG infested oat inoculum. The inoculum was placed next to the taproot of the plants 1 to 2 cm below the soil surface at V7 to V9 growth stage. They concluded that inoculation with FSG infested oats is a reliable alternative to the use of naturally infested soil when soybean cultivars are evaluated for field reactions to FSG. Ringler (1995) compared four inoculation methods and concluded that inoculation with FSG infested

sorghum placed next to the taproot of the seedlings at the V2 stage was effective for screening cultivars for SDS resistance, but was tedious to apply. The author emphasized the need to identify methods for conducting SDS field studies.

Greenhouse resistance assays have been done in several SDS resistance studies, by using FSG infested mixture of oat (Lim and Jin, 1991; Stephens et al., 1993), sorghum (Hartman et al., 1997; Mueller, 2001), cornmeal (Killebrew et al., 1988) or toothpicks (Klingesfuss et al., 2002). However, according to Torto et al. (1996), the existing greenhouse assays fail to predict accurately the field responses of genotypes to SDS. This may be in part because the inoculum rates in greenhouse tests are generally higher (more than 10,000 spores cm^{-3} of plant growth medium) than in the field (less than 5,000 spores cm^{-3} of plant soil) (Torto et al., 1996; Roy et al., 1997). The high inoculum rates may overcome resistance of soybean to FSG resulting in poor correlations with field results (Torto et al., 1996).

Greenhouse evaluations have been carried out to study the genetic control of resistance (Stephens et al., 1993) and to identify loci and alleles that underlie resistance to SDS (Fronza et al., 2002). Nijti et al. (2001) compared selection for field resistance to SDS in the greenhouse at varying rates of FSG infested sorghum inoculum. The moderate inoculum rate of 4,000 spores cm^{-3} of plant growth medium resulted in the best correlation (0.77) with the field results. Hartman et al. (1997), screened cultivars and plant introductions for SDS resistance in a greenhouse using FSG infested sorghum applied in the soil. Although no correlations were taken with field results, the germplasm classified as resistant to SDS in the greenhouse were confirmed as resistant in field experiments.

The high cost and large number of locations for evaluating SDS resistance in the field supports the use of marker-assisted selection as a valuable selection tool for plant breeders in the

development of SDS resistant cultivars. Hnetkovsky et al. (1996), working with recombinant inbred lines (RIL) developed from a cross between the SDS resistant cultivar Forrest (Hartwig and Epps, 1973) and the SDS susceptible cultivar Essex (Smith and Camper, 1973) identified two SDS resistance alleles from quantitative trait loci (QTL) located on linkage group (LG) G that were derived from Forrest. These QTL jointly accounted for 34 % of the total phenotypic variability of SDS disease incidence (DI). Later, Iqbal et al. (2001), working with the same population, defined two more resistance QTL alleles derived from Forrest. Jointly the four QTLs explained 50 % of the variation on DI. Additionally, the authors found that two QTL on LG I and LG C2, which derived their beneficial alleles from Essex and jointly explained about 40 % of the variation in SDS DI. The resistance from all six loci jointly explained about 91% of the variation for SDS resistance in the population**.** The authors suggest that cultivars with durable resistance to SDS can be developed via gene pyramiding.

Nijti et al. (2001), working with recombinant inbred lines from a cross between the SDS resistant cultivar Pyramid (Myers and Schimdt, 1988) and the SDS susceptible cultivar Douglas (Nickel at al., 1982) identified two SDS resistance QTL alleles derived from Pyramid. These alleles were located in the same region on LG G where SDS resistance QTL were previously identified in Forrest. A third QTL with the resistance allele from Douglas was also mapped in this population. This QTL mapped to the same region on LG C2 that a SDS resistance QTL allele had been previously identified in Essex.

Additional research is needed to explore the nature of resistance of SDS, identify new sources of resistance and establish new and better ways to evaluate and incorporate SDS resistance on elite cultivars (Roy et al., 1997; Muller, 2001). The identification of new loci with distinct mechanisms of resistance plays an important role for using gene pyramiding as an

effective method for developing cultivars with stable SDS resistance (Nijti et al., 1998; Nijti et al., 2001).

The objectives of my research were to (i) identify an effective FSG inoculation method for field evaluation of cultivars or segregating populations for reaction to SDS, (ii) evaluate the effect of compaction and irrigation timing on the occurrence of SDS symptoms, (iii) determine how well two greenhouse inoculation methods correlate to field resistance to SDS, (iv) evaluate soybean lines developed from crosses between Ina (Nickell et al., 1999) and LN 91-1695, and PI 567374 and Omaha (Nickell et al., 1998) for resistance to SDS and (v) to detect QTLs associated with resistance to SDS.

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CHAPTER ONE

EFFECT OF *Fusarium solani* f. sp. g*lycines* INOCULATION METHODS, COMPACTION AND IRRIGATION TREATMENTS ON THE OCCURRENCE OF SOYBEAN [*Glycine max* (L.) Merr.] SUDDEN DEATH SYNDROME

ABSTRACT

Field studies measuring the reaction of soybean [*Glycine max* (L.) Merr.] cultivars to sudden death syndrome (SDS), caused by the fungus *Fusarium solani* f. sp. g*lycines* (FSG), typically have been conducted in fields with histories of SDS symptoms. However, the occurrence of SDS in a field is unpredictable and the disease is often not present when the experiments are conducted**.** The objective of this study was to evaluate the effect of field inoculation methods, soil compaction, and irrigation timing on the occurrence of SDS symptoms. Experiments to evaluate inoculation methods were conducted in Urbana, IL in 2002 and 2003, in fields with no history of high SDS incidence. Six treatments which included FSG infested grain of white sorghum [*Sorghum bicolor* (L.) Moench], popcorn (*Zea mays everta*) or oat (*Avena sativa* L.) were planted in the furrow with the soybean seed, broadcasted and incorporated into the soil prior to planting or placed below the soybean seed just prior to planting. Three experiments were conducted in Urbana, IL in 2002, 2003 and 2004 to evaluate the effect of compaction and irrigation on SDS symptom occurrence. These experiments were planted in fields with no history of high SDS infections and inoculated with FSG infested sorghum grain prior to planting. The compaction treatments were done by driving a truck across the field once in early spring. Irrigation treatments were applied with a trickle irrigation system. The six

irrigation treatments included natural rain and combinations of irrigation application at V3, V7, R3, R4 and/or R5 growth stages. Disease incidence (DI) disease severity (DS) and disease index (DX; DI*DS/9) were determined to evaluate foliar SDS symptoms along with seed yield, days to maturity, plant height and lodging. The inoculation methods that produced the most severe foliar symptoms included placing infested sorghum below the seed prior to planting (DX=36.1) and planting infested popcorn in the furrow with the soybean seed (DX=28.7). A significant ($P<0.0001$) linear relationship was observed between yield and DX ($y = 3738.4 – 23.1078x$) across years. The correlations between DX and yield were -0.86 in 2002 and -0.61 in 2003. No significant effects of soil compaction on SDS foliar symptoms development were observed. We observed that irrigation treatments during mid to late reproductive growth stages were an important factor for increasing SDS foliar symptom development.

INTRODUCTION

Sudden death syndrome (SDS) is a mid to late season fungal disease caused by *Fusarium solani* f. sp. g*lycines* (FSG), which can cause severe yield losses to soybean [*Glycine max* (L.) Merr.] (Gibson et al., 1994). The fungus is soilborne and infects plants through the roots resulting in a reduction of both root mass and number of viable root nodules. The above ground symptoms include interveinal chlorosis and necrosis of leaves, premature defoliation, and pod abortion (Hartman et al., 1997). The foliar symptoms develop rapidly usually during the reproductive stage of soybean growth (Gibson et al., 1994).

The use of resistant cultivars is the most effective method for controlling SDS. Some cultivars and lines with good levels of resistance have been identified (Hartman et al., 1997; Hartwig et al., 1996; Schmidt et al., 1999; Mueller, 2001) and this resistance has been shown to

be polygenic in field studies (Hnetkovsky et al., 1996; Chang et al., 1996). For example, resistance was conditioned by a minimum of five loci in a population developed from crossing "Forrest" with "Essex" (Meksen et al., 1999). Resistance to SDS is also partial since all soybean genotypes tested have shown some SDS symptoms under severe disease pressure (Nijti et al., 1996; Igbal et al., 2001).

Field studies measuring the reaction of cultivars to SDS typically have been conducted in fields with histories of SDS symptoms (Nijti et al., 1996). However, selection for SDS resistance in the field is difficult because of the sensitivity of symptom development to environmental factors (Gibson et al., 1994; Nijti et al., 1996). The occurrence of SDS in a field is unpredictable and the disease is often not present when the experiments are conducted**.** Artificial inoculation methods have been used; however, the efficiency of these methods is not clear. Stephens et al. (1993) used microplots to evaluate reactions of 12 soybean cultivars grown in soil both naturally infested and inoculated with FSG. Plants were inoculated at the V7 to V9 growth stage (Fehr and Calviness, 1971) by placing 15 oat infested grains next to the taproot 1 cm below the soil surface. They concluded that the inoculation of soil with FSG infested oats is a reliable alternative to the use of naturally infested soil when soybean cultivars are evaluated for field reactions to FSG. Ringler (1995) compared four field inoculation methods and concluded that FSG infested sorghum seeds placed next to the taproot at the V2 growth stage was effective for screening cultivars for SDS resistance, but was tedious. The author emphasized the need to identify methods for conducting SDS field studies.

The association of high soil moisture and soil compaction with greater occurrence and severity of SDS is a common field observation (Ringler, 1995; Roy et al., 1997). Melgar et al. (1994) reported that the incidence and severity of SDS was greater in irrigated than non-irrigated

plants. Scherm and Yang (1996), reported a positive association between soil moisture and SDS severity, showing that soil moisture plays an important role in the relationship between SDS severity and yield loss. In the greenhouse, Roy et al. (1989) also found a positive relationship between soil moisture and SDS incidence. However, information on the optimal timing of irrigation to promote SDS symptom development is not available.

Sudden death syndrome symptoms have been found to increase in compacted areas of fields when compared to non-compacted areas. This trend may be caused by compacted soils being wetter than non-compacted soils. Deep tillage in poorly drained fields to break up compaction can reduce SDS occurrence (Hartman et al., 1999). Vick et al. (2003) compared tilled plots with no-till plots and observed that subsoiling dramatically reduced symptoms of SDS. The authors concluded that in areas where SDS occurs and soil compaction exists, subsoiling can be used to reduce the severity of SDS foliar symptoms.

The development of a reliable field inoculation method as well as greater knowledge about the effect of moisture and soil compaction on SDS symptom development will help researchers identify genotypes with resistance to the disease. The objective of this study was to evaluate the effect of field inoculation methods, soil compaction, and irrigation timing on the occurrence and development of SDS symptoms.

MATERIAL AND METHODS

Inoculation methods experiment

The experiments were conducted in Urbana, IL in 2002 and 2003 in fields with no history of high SDS incidence. Main plots were arranged in a randomized complete block design with four replicates. Main plots were divided in a split-plot, with inoculation methods applied to

main plots and cultivars to subplots. The experiment was sowed on 10 June in 2002 and 1 May in 2003. The soil type of the field during both years was a Elburn silt loam (fine-silty, mixed, superactive, mesic Aquic Argiudolls). Each cultivar in a subplot was sown in a 4-row plot that was 5 meters long with a 75 cm row spacing, a planting depth of 3 cm and a seeding rate of 350,000 seeds ha^{-1}. The cultivars used were Asgrow AG 3302, which is classified as partially resistant and Asgrow AG 3003, which is classified as susceptible (Monsanto, 2005). The inoculation methods evaluated in 2002 were: 1) no inoculum (control), 2) infested sorghum (300 kg ha^{-1}) broadcasted and incorporated into the soil prior to planting, 3) infested popcorn (40 kg ha^{-1}) planted in the furrow with the soybean seed, 4) infested oats (120 kg ha^{-1}) broadcasted and incorporated into the soil prior to planting, 5) liquid inoculum placed 5 cm below the seed prior to planting (500 l ha^{-1}), and 6) infested sorghum (45 kg ha^{-1}) placed below the soybean seed just prior to planting. For inoculation methods 2 and 4, the infested grain was broadcasted with a fertilizer drop spreader and incorporated with a rear tine rotor tiller to a depth up to 8 cm. For inoculation method 3, the inoculum was mixed with the seed just prior to planting. For inoculation method 6, the infested sorghum seeds were planted with the plot planter at a depth of 8 cm. The planter was then reset to a normal depth, and the soybean seeds were planted directly on top of the infested sorghum. The Monticello isolate of FSG (originating from Monticello, IL) was used to produce all the inoculum. The grain inoculum was prepared by first soaking seed of white sorghum [*Sorghum bicolor* (L.) Moench], popcorn (*Zea mays everta*) or oat (*Avena sativa* L.) overnight. Four kg of seeds were placed into a clear autoclave bag and autoclaved for 1 hour twice. Each bag was then inoculated with 30 plugs (4 mm diameter) of fungal mycelium and incubated at room temperature for two weeks. For the liquid inoculum, one 100 x 15 mm plate of ground mycelium per liter of water was used. The colony forming unit (CFU) of the infested

seeds was determined as previously reported on hairy roots (Li et al., 2002) with slight modification. Briefly, 1 g of sorghum inoculum was soaked in a 250-ml Erlenmeyer flask containing 100 ml of sterile distilled water. The flasks were shaken at 150 rpm on an Orbital Shaker for 30 min, and then the mixture was serially diluted 10 fold with sterile distilled water twice. From each dilution, 100 µl of inoculum dilution was spread on an agar plate (100 x 15 mm) containing *Fusarium solani* f. sp. *glycines* semi-selective medium (Huang and Hartman, 1996). Six plates were used for each inoculum dilution. The plates were incubated at room temperature (25 ± 2ºC) for 10 days. Colonies of *Fusarium solani* f. sp. *glycines* were identified as described previously (Li et al., 2000). The number of colonies on each plate was used to determine the colony-forming units per gram of sorghum. The experiment was conducted two times.

To observe the residual effect of the inoculum in the soil, the same cultivars were planted in 2003 in the same positions in the field as the 2002 experiment, without any re-inoculation. This experiment was analyzed as a split-split-plot design, with inoculation methods applied to main plots, cultivars as subplots and years as sub-subplots. In the inoculation and residual effect experiments, approximately 76 mm of water was applied to the plots at the growth stages V3, V7 and R4 using overhead irrigation to provide sufficient soil moisture to favor disease development.

Compaction/irrigation experiments

Experiments were conducted in Urbana in 2002, 2003 and 2004 in fields with no history of high SDS infections. The soil type of the field during all three years was a Elburn silt loam (Fine-silty, mixed, superactive, mesic Aquic Argiudolls). All plots in these experiments were inoculated with infested sorghum grain by placing the inoculum below the soybean seed

according to inoculation method 6. The experimental unit was an 8-row plot, 5-meters long with a 75 cm row spacing. The seeding rate was 350,000 seeds ha^{-1} and the planting depth was 3 cm. In all three years, four replications of a randomized complete block design were used. In 2002, the plots were arranged in a split-split plot design. The compaction treatments were the main plots, irrigation treatments were the sub-plots and cultivars were the sub-sub-plots. The 2002 experiment was sowed on 16 May, the 2003 experiment was sowed on 1 May, and the 2004 experiment was sowed on 29 April. The compaction treatments were done by driving a truck across the field once in early spring. The compaction was measured with a Rimik CP20 cone penetrometer (Agridy, Rimik, Toowoomba, Queensland, Australia) 30^{o} cone tip with a base diameter of 1.27 cm at the growth stage V3.

Irrigation treatments were applied with a trickle irrigation system. The irrigation tapes were placed next to the two center rows of each plot, and the equivalent to 76 mm of rain was applied to the two center rows of the plots at each irrigation application. The 2002 and 2003 irrigation treatments were as follows: 1) natural rain (control), 2) natural rain with irrigation at V3, 3) natural rain with irrigation at V7, 4) natural rain with irrigation at V3 and V7, and 5) natural rain with irrigation at V3, V7 and R4.

The experiment was repeated in 2003 and 2004 without the compaction treatment. During these years, plots within blocks were arranged in split-plot, using irrigation treatments as main plots and cultivars as subplots. In 2004, the following irrigation treatments were applied: 1) natural rain (control), 2) natural rain with irrigation at R4, 3) natural rain with irrigation at V3 and V7, 4) natural rain with irrigation at V3, V7 and R4, 5) natural rain with irrigation at V3, V7, R4 and R5, and 6) natural rain with irrigation at R3, R4 and R5. The treatments were

applied to the cultivars AG 3003 and AG 3302, which also were used in the inoculation experiment.

SCN egg counts

For all experiments, soil samples were taken just prior to harvest to measure the level of SCN infestation. Each sample consisted of ten sub-samples of 20 cm deep cores. In each replicate from each experiment, a sample was taken from the control plot and from the treatment which showed the most severe SDS symptoms. A 100 cm^3 sub-sample of soil was taken from each sample and processed according to Bird et al. (1976)**.** Briefly, a 100 cm^3 sub-sample of soil was taken from each sample and processed using a semi-automatic elutriator to extract the cysts from the soil (Univ. of Georgia Science Instrument Shop, Athens, GA). The cysts were grounded with a rubber stopper to release the eggs on nested 150 Φm over 75 Φm over 25 Φm wire mesh sieves. The egg suspension was then stained with 3 ml of egg staining solution (0.35g of Acid Fuschion, 250 ml Lactic Acid, 750 ml water) and microwaved on high temperature until the sample had boiled for at least 30 seconds. The sample was diluted with water by bringing the volume to 100 ml. A 5 ml sample was counted to estimate the number of eggs of *Heterodora glycines* in each plot.

Collection of field data

For all experiments, research plots were rated for maturity date, plant lodging, plant height, seed yield, and SDS foliar symptoms. Maturity date was taken as the date when 95% of the pods had turned to their mature color. Plant lodging was rated at R8 on a scale from 1 = all plants upright to 5 = all plants prostrate, and plant height was measured in cm at R8 as the distance from the ground to the uppermost node of an average plant. Seed yields were measured

by harvesting the center two rows of plots and were reported as kg/ha^{-1} on a 13% moisture base. The disease incidence (DI) and disease severity (DS) were taken according to Gibson et al. (1994) at the R6 growth stage. Disease incidence was taken as a percentage of plants with foliar symptoms. Foliar disease severity was recorded as: 1= 0 to 10% chlorosis or 1 to 5% necrosis, 2 = 10 to 20% chlorosis or 6 to 10% necrosis, 3 = 20 to 40% chlorosis or 10 to 20% necrosis, 4 = 40 to 60% chlorosis or 20 to 40% necrosis, 5 = > than 60% chlorosis or > than 40% necrosis, 6 = up to 33% defoliation, 7 = up to 66% defoliation, 8 = > than 66% defoliation and 9 = premature death of the plant. A disease index (DX; 0-100) was calculated as (DI x DS)/9.

Statistical analysis

For all experiments, analysis of variance was computed for the field data using the mixed procedure of SAS (SAS Institute, 2000) with standard analysis methods for a split-split plot and split-plot test (Snedcor and Cochran, 1980). Years and blocks were treated as random factors, while all other factors were treated as fixed. The exception was the residual effect experiment, where years were also considered a fixed factor. Means were separated using LSD (5%). Normality and homogeneity of variances of the data were verified**.** The CORR procedure of SAS (SAS Institute, 2000) was used to calculate correlations between traits.

RESULTS AND DISCUSSION

Inoculation experiments

Low SDS symptoms were observed for non-inoculated control plots in 2002 and 2003, indicating that the nurseries had minimal natural infestation of FSG (Table 1.1). Typical root and foliar symptoms of SDS were observed for some inoculation methods. Results from SCN eggs counts (Table 1.2) showed low to moderate end of season egg densities, indicating that SCN was not a major factor in these field tests. There were sufficient colony forming units (CFU) from the

inoculum used in the 2003 experiment (Table 1.3) to indicate that all grain were efficient in multiplying the FSG.

Inoculation methods had a significant effect ($P< 0.05$) on DX and maturity. Significant differences between cultivars were observed for DX and yield. A significant inoculation methods x cultivar interaction was detected for DX. No significant effects were observed for lodging and plant height (Table 1.4).

The SDS symptom data obtained in 2002 and 2003 (Table 1.1) were very consistent with no changes in the ranking among inoculation methods. As expected, more severe SDS symptoms were observed for the susceptible cultivar AG 3003 than for partially the resistant cultivar AG3302 (Table 1.5). The average DX for AG 3003 was 13.4, which was significantly greater than the average DX of 3.4 observed for AG 3302. No significant differences for DX were observed among the inoculation methods for AG 3302. Significant differences for DX were observed between the cultivars AG 3003 and AG 3302 for methods 3 and 6 (LSD=7.6). This shows that these methods were effective in separating the partially resistant cultivar from the susceptible cultivar. For the susceptible cultivar AG 3003, both inoculation methods 6 (DX=36.1) and 3 (DX=28.7) resulted in significantly greater DX than the control (DX=3.1). Across years (Table 1.1), DX values significantly greater than the control were observed for inoculation methods 6 and 3, while DX values that were not significantly different from the control were observed for all the other treatments. The inoculum was placed close to the seed for treatments 6 and 3, which is the likely reason for the high DX values. In contrast, much lower levels of disease were observed for methods 2 and 4, where the inoculum was broadcasted and incorporated into the soil prior to planting. In addition, the oat seed used in method 4 are bulky and fibrous making it difficult to administer consistent dosage of inoculant (Ringler, 1995).

Method 5 (liquid inoculum) showed practically no symptoms, indicating that there is a need of a carrier in the process of field inoculation.

Except for method 6, no significant differences in yield from the control were detected for the partially resistant cultivar AG 3302 (Table 1.5). For AG 3003, significantly lower yields than the control were observed for both treatments 3 and 6. The significantly lower yields were likely the result of SDS, as inoculation methods 3 and 6 caused the greatest symptoms expression. For AG 3003, a significant ($P< 0.0001$) linear relationship was observed between yield (Y, Kg ha^{-1}) and DX ($Y = 3738.4 – 23.10DX$, Figure 1.1). The yield losses for these treatments were 6.7% and 7.5% to each increase in 10 DX units. Yield losses caused by SDS have been reported previously (Rupe et al., 1993; Hartman et al., 1999). The results presented in my paper are similar to those observed by Luo et al. (2000). The authors observed that an increase in each DX unit caused a yield loss from 18 to 29 kg ha^{-1}. However, in other studies slight yield losses due to SDS were reported (Hersmann, 1988; Stephens et al, 1993). This lack of correlation between yield and SDS symptoms might be related to the differential tolerance of cultivars to the disease. Timing of symptom development is also an important factor in yield losses. The effect of SDS on yield depends on the growth stage of the host at the onset of symptom development, and whether the disease progresses rapidly and becomes severe (Roy et al., 1997). Stephens et al. (1993) observed that for SDS to affect yield, the disease must become severe before R5. In my studies the first symptoms were, in general, observed at R4. The strong negative association between DX and yield is evident by the significant correlations between these traits (Table 1.6).

Due to premature defoliation caused by the disease, plants in plots treated with inoculation method 6 reached maturity significantly earlier than plants in the control plots (Table

1.1). This maturity difference was modest, with inoculation method 6 maturing three days earlier than the control. The correlation values between DX and maturity were significantly negative. No significant inoculation method or cultivar effects were detected for plant height.

Inoculation residual effect experiment

The overall SDS mean score for the 2003 residual effect experiment was 9.2, similar to the score of 7.6 observed for the 2002 experiment (Table 1.7). The DX scores for the susceptible cultivar AG 3003 were similar in 2002 and 2003. However, the analysis of variance (Table 1.8) indicated a significant year x inoculation methods interaction. The analysis done by inoculation methods (Table 1.9) showed significant effects of year for inoculation method 2 (infested sorghum broadcasted and incorporated prior to planting) and inoculation method 6 (infested sorghum placed below the seed just prior to planting).

Increase in DX for AG 3003 between years in the residual effects experiment was observed for method 2. The DX went from 4.1 in 2002 to 16.6 in 2003 (Table 1.7). The sorghum broadcasted and incorporated prior to planting may have resulted in the placement of the inoculum far enough from the soybean seed to escape symptom causing infection in 2002. However, sufficient infection occurred in 2002 to result in multiplication of the inoculum causing infection in 2003.

Conversely, a significant decrease in SDS foliar symptoms was observed for inoculation method 6 from 2002 to 2003. In 2002, cultivar AG 3003 showed a DX of 38.3, while in 2003 is this rate decreased to 22.2 (Table 1.7). One possible explanation is that the soybean seed were not planted during 2003 in exactly the same position as 2002. Despite this decrease in DX in 2003, the observed DX rates are sufficient to successfully screen genotypes for resistance. The decision to apply the inoculum every year depends on the available infra-structure of the research

program. However, the production of the inoculum is relatively simple and the field application every year would be a good choice. Consistent with the previous inoculation experiments, a negative correlation of –0.83 between DX and yield for AG 3003 was observed for the residual experiment.

In summary, I found that inoculation methods 6 (infested sorghum placed below the seed) and 3 (infested popcorn planted in the furrow with the soybean seed) produced the most severe foliar symptoms and can be used for screening genotypes in field experiments. In addition these methods can be easily applied in large fields, in contrast to other methods such as described by Stephens et al. (1993) and Ringler (1995). Further experiments should be conducted to identify the optimum inoculum rates.

Irrigation experiment

Typical SDS root and foliar leaf symptoms were observed in the irrigation experiment during all three years. Low end of season SCN egg densities were observed from egg counts in each year (Table 1.2). The soil compaction treatments resulted in significant increases in compaction (Table 1.10).

The analysis of variance of the 2002 experiment indicated that the effect of compaction was significant only for yield. Across cultivars, the non-compacted treatment yielded 4064 kg ha^{-1}, which was significantly greater than 3725 kg ha^{-1} observed for the compacted treatment. Soil compaction impedes root development, decreasing the plant's ability to take up nutrients and water. This, in turn can cause yield losses, especially if rainfall is limited during the reproductive stages (Nogueira and Manfredini, 1983). Since the effect of compaction was not significant for DX, this treatment was not included on the 2003 and 2004 experiments.

The analysis of variance across 2002 and 2003 (Table 1.11) showed no significant effect of irrigation treatments for all traits across the two cultivars. However, a significant irrigation treatment x cultivar interaction was detected for yield. Even though the irrigation treatment x cultivar interaction was not significant for the other traits, contrasts for irrigation treatments were calculated by cultivar for those traits, because of the high SDS susceptibility for AG 3003. Therefore, the results will be discussed by cultivar.

The overall mean across two years (Table 1.12) was 6.7 for DX, 4253 kg ha^{-1} for yield and 122.8 days for maturity. When DX values were analyzed separately by cultivar, no significant differences for any trait were detected among the irrigation treatments for the partially resistant cultivar AG 3302 (Table 1.13). For the susceptible cultivar AG 3003, irrigation treatment 5 produced significantly more severe symptoms than irrigation treatments 1, 2 and 3. These results support previous results (Hirrel, 1987; Roy et al., 1989; Melgar et al., 1994; Scherman and Yang, 1996) that SDS is favored by high soil moisture. According to Scherman & Yang (1996) the most severe SDS foliar symptoms would be expected after the appearance of favorable conditions during early stages for root colonization and infection followed by favorable conditions (such as high moisture and intermediate to high temperature) for plant growth. This would lead to high translocation of toxins from the roots to the foliage.

The results obtained in my work showed especially the importance of irrigation during the R4 stage in causing SDS symptoms. The dramatic response to irrigation at R4 suggests that water is important in the translocation of toxins from the roots to the foliage. Another hypothesis is that reduced soil aeration could cause higher toxin production by the fungus. Miller and Burke (1977) implicated reduced soil aeration as a predisposing factor for bean root rot in wet soils. In contrast, no significant increase in SDS symptoms was observed for the other irrigation

treatments applied during vegetative stages compared to the control. Some authors have indicated the importance of high soil moisture at early stages on the development of SDS foliar symptoms in soybeans. Rupe (1988) isolated FSG from soybean roots as early as 3 weeks after planting in the field indicating that early season infection may be important for later SDS symptom development. Roy et al. (1989) found that SDS symptoms were more severe in plants irrigated continuously from V3 stage compared with those irrigated continuously from V8 stage, indicating that high soil moisture, probably early in soybean development, is critical for SDS symptom development. The lack of response of the irrigation treatments during vegetative stages in my experiments may indicate that there was sufficient soil moisture during these stages to enable good root colonization and infection of FSG on the plant roots.

Analysis done for the cultivar x irrigation treatment interaction showed no significant effects of irrigation treatments on yield for either cultivar (Table 1.13) or across cultivars (Table 1.12). Treatment 5, which caused the greatest DX, showed no significant yield losses. The SDS caused by the additional irrigation at the R4 stage might have been compensated by the increase in yield that resulted from the irrigation treatment promoting greater pod fill.

Due to premature defoliation caused by the disease, maturity was significantly earlier for AG 3003 receiving irrigation treatment 5 compared to the control. No significant differences among treatments were observed for lodging and plant height.

The analysis of variance of the 2004 irrigation experiment (Table 1.14) indicated significant effect of irrigation treatments only for maturity. Significant differences between the cultivars were observed for all traits, except plant height, while a significant cultivar x irrigation interaction was detected only for yield. Similar to the previous experiments, the results will be discussed by cultivar due to the contrasting SDS reactions of the cultivars.

Significant effects of irrigation treatments were observed only for the susceptible cultivar AG 3003 in 2004 (Table 1.15). This cultivar showed significantly greater DX for three treatments that included irrigation during reproductive stages when compared to the control. One irrigation at R4 stage (treatment 2) resulted a significant increase in SDS foliar symptom, while the irrigation applied only at V3 and V7 vegetative stages (treatment 3) was not effective in significantly increasing DX rates compared to the control. However, the greater DX rates were detected for irrigation treatments 4 (DX= 26.3) and 5 (DX=30), showing a tendency of higher DX rates with water application during both vegetative and reproductive stages of the plants.

Similar to the previous experiments, no effect of irrigation was detected for yield, and the most diseased plants of treatments 2, 4 and 5 reached the maturity significantly earlier than the control. No effect of irrigation was observed for plant height and lodging.

In summary, there were no significant effects of soil compaction on SDS foliar symptoms development, but compaction caused a significant yield decrease. On the other hand, I observed that moisture, especially during mid to late reproductive growth stages, is an important factor for SDS foliar symptom development. Even in 2002 and 2004 (Figures 1.2, 1.3 and 1.4), when good rainfall occurred during late reproductive stages R4 to R6, the response of SDS symptoms to irrigation was significant. One limitation of my study was that I did not control natural rain. The lack of significant effects from irrigation treatments during vegetative growth stages may be because of the rain received during these stages. If less rain occurred, perhaps irrigation treatments during vegetative growth stages would have had a significant effect. Further studies are needed to more accurately identify optimum water rates for SDS foliar symptom development.

Table 1.1. Means of disease index (DX), seed yield and maturity for two soybeans cultivars tested with six *Fusarium solani* f. sp.g*lycines* inoculation methods during 2002 and 2003.

	DX			Yield (Kg ha^{-1})			Maturity (days)		
Inoculation method	2002	2003	Mean	2002	2003	Mean	2002	2003	Mean
Sorghum below seed (6)	25.6	20.0	22.8	3303	3051	3177	112.6	126.2	119.4
Popcorn furrow (3)	14.9	19.7	17.3	3628	3105	3367	113.9	126.5	120.9
Sorghum broadcasted (2)	3.0	8.7	5.9	4016	3064	3540	115.6	126.5	121.2
oats broadcast (4)	1.9	2.0	2	4104	3652	3878	116.4	127.5	121.8
Control (1)	0.0	4.5	2.2	4220	3505	3863	116.6	128.5	122.5
Liquid Inoculum (5)	0.0	0.3	0.1	4396	3388	3892	116.7	128.5	122.5
Overall mean	7.6	9.2	8.4	3944	3294	3619	115.2	127.2	121.2
LSD (5%) †	7.5	8.6	7.7	748.3	534.9	843	1.8	2.3	1.8

† Least significant difference at P = 0.05.

Table 1.2. Average number of soybean cyst nematode (SCN) eggs/100cm^3 of soil, for the compaction/irrigation experiment (C/I) across three years, for the inoculation method experiment (inoculation) across two years and for the residual experiment in 2003.

Experiment	2002	2003	2004
	----------SCN eggs/100cm^3 of soil----------		
C/I	19	75	47
Inoculation	154	468	---
Inoculation residual	---	413	---

Table 1.3. Colony forming units (CFU) from samples of sudden death syndrome infested sorghum, oats and popcorn seeds used in 2003.

Inoculum	CFU/g inoculum †
Sorghum	2.4×10^5
Oat	2.2×10^5
Popcorn	3.5×10^3

†Mean of three replicated plates from two CFU samples

Table 1.4. Sources of variation, degrees of freedom (df), mean squares, and significance values for disease index (DX), seed yield, maturity, plant height and lodging for two soybeans cultivars and six *Fusarium solani* f. sp. g*lycines* inoculation methods across 2002 and 2003.

		Mean Square				
Source of variation	df	DX	Yield	Maturity	Plant height	Lodging
Year	1	64.0 ^{ns}	10141973**	3408.1***	1881.5 ^{ns}	33.2 ^{ns}
Rep(year)	6	202.0	553102	93.3	34.3	0.9
Inoc	5	1409.0**	1493304 ^{ns}	132.5*	4.2 ^{ns}	0.5 ^{ns}
Inoc x rep(year)	30	132.3	194909	97.2	22.7	0.7
Year x Inoc	5	73.1 ^{ns}	349739 ^{ns}	24.8 ^{ns}	4.8 ^{ns}	0.4 ^{ns}
Cultivar	1	2403.3 **	3509938*	32.6 ^{ns}	504.2 ^{ns}	9.0 ^{ns}
Cultivar x year	1	29.2 ^{ns}	3641.7 ^{ns}	13.5*	176.1***	0.4 ^{ns}
Cultivar x inoc	5	536.2***	169178 ^{ns}	9.8 ^{ns}	32.1 ^{ns}	0.4 ^{ns}
Cultivar x year x inoc	5	10.0 ^{ns}	48779 ^{ns}	5.5 ^{ns}	3.4 ^{ns}	0.3 ^{ns}
Residual	36	48.3	32440	1.4	12.4	0.2

*, **, ***,ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 1.5. Means of disease index (DX), seed yield and maturity for two soybean cultivars using six *Fusarium solani* f. sp. *glycines* inoculation methods across 2002 and 2003.

Inoculation Methods	Disease Index (DX)		Yield (Kg ha^{-1})		Maturity (days)	
	AG3003	AG3302	AG3003	AG3302	AG3003	AG3302
Sorghum below seed (6)	36.1	9.5	2932	3422	118.6	120.1
Popcorn in furrow (3)	28.7	5.9	3022	3711	119.1	121.2
Sorghum broadcasted (2)	8.8	2.9	3425	3656	120.2	121.8
Oats broadcasted (4)	3.3	0.7	3794	3962	121.3	122.2
Control (1)	3.1	1.2	3751	3975	122.2	122.6
Liquid Inoculum (5)	0.3	0	3646	4139	122.4	122.6
Mean	13.4	3.4	3429	3811	120.7	121.8
LSD 5% †	11.6	11.6	537	537	2.1	2.1

† Least significant difference at P = 0.05.

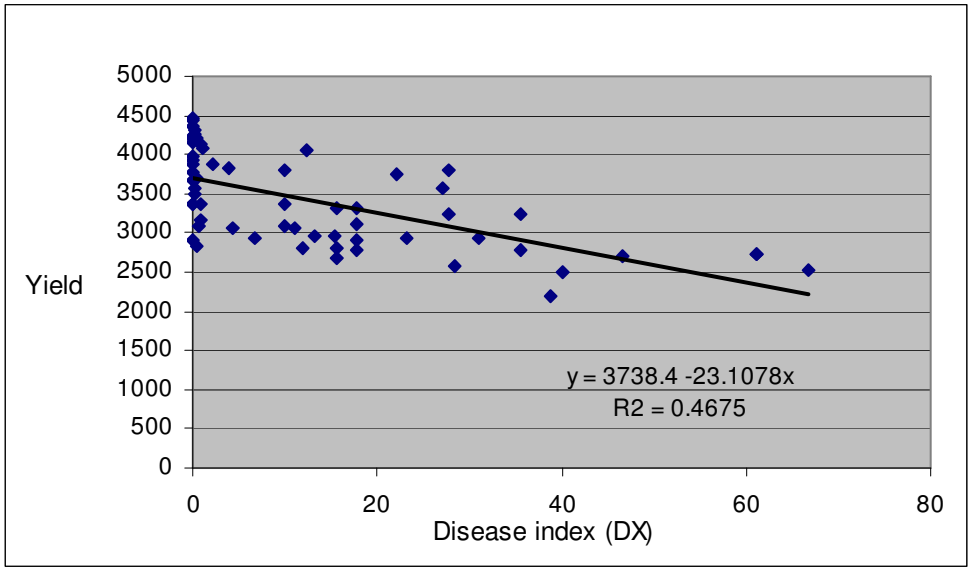

Figure 1.1. Regression line for sudden death syndrome disease index (DX) with seed yield (kg/ha^{-1}) for susceptible cultivar AG 3003.

Table 1.6. Correlation coefficients between disease index (DX), yield and maturity for the cultivar AG 3003, across six *Fusarium solani* f. sp. g*lycines* inoculation methods.

	DX		Yield		Maturity	
Trait	2002	2003	2002	2003	2002	2003
DX	-----	-----	-0.86***	-0.61***	-0.88**	-0.44*
Yield	-0.86***	-0.61***	-----	-----	0.82***	0.32ns

*, **, ***,ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 1.7. Residual effects of 2002 field inoculations on 2003 means of disease index (DX) and yield for two soybeans cultivars using six *Fusarium solani* f. sp. g*lycines* inoculation methods.

	DX				Yield (Kg ha^{-1})	
	Across cultivars		AG 3003			
Inoculation methods	2002	2003	2002	2003	2002	2003
Sorghum below seed (6)	25.6	14.4	38.7	22.2	3013	2872
Popcorn furrow (3)	14.9	17.9	26.4	23.3	3208	3202
Sorghum broadcasted (2)	3.0	12.8	4.1	16.6	3903	3117
Oats broadcasted (4)	1.9	6.4	3.4	11.7	4004	3134
Control (1)	0.0	0.2	0.0	0.4	4194	3724
Liquid Inoculum (5)	0.0	0.1	0.0	0.3	4160	3777
Overall mean	7.6	9.2	12.0	12.4	3747	3304
LSD 5% †	7.5	5.9	16.5	6.8	562	606.2

† Least significant difference at P = 0.05.

Table 1.8. Sources of variation, degrees of freedom (df), mean squares, and significance levels for the disease index (DX) of soybean cultivars using six *Fusarium solani* f. sp. g*lycines* inoculation methods in the residual experiment in 2002 and 2003.

	DX	
Source	df	Mean Square
Inoculation methods	5	1070.97***
Cultivar	1	1582.76***
Inoc*cultivar	5	280.60**
Year	1	21.34^{ns}
Rep(year)	6	108.26^{ns}
Ino*rep(year)	30	114.67 ^{ns}
Year*inoc	5	198.70*
Year*cultivar	2	12.51^{ns}
Year*cultivar*inoc	5	67.00^{ns}
Residual	35	30.26

*, **, ***, ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 1.9. Sources of variation, degrees of freedom (df), mean squares, and probability values in tests of the effect of year on disease index (DX) in the residual effects experiment on two soybean cultivars for each inoculation methods in 2002 and 2003.

Inoculation methods	df	Mean Square	F value	Pr > F
Control (1)	1	0.2	0.00	0.9412
Sorghum broadcasted (2)	1	381.1	5.3	0.0075
Popcorn furrow (3)	1	37.2	0.5	0.3679
Oats broadcasted (4)	1	78.0	1.1	0.1986
Liquid Inoculum (5)	1	0.1	0.0	0.9622
Sorghum below seed (6)	1	526.4	7.3	0.0082

Table 1.10. Means of soil compactedness of compacted and non compacted treatments at three soil depths in 2002.

Treatment	Depth (cm)		
	0-10	10-20	30-60
	----------Kpa[+]----------		
Compacted	1821	1379	1517
Non compacted	1055	1053	1423
LSD (5%)	479	235	116

[+]Kpa, mean of 4 replications, 100 measurements/replication

Table 1.11. Sources of variation, degrees of freedom (df), mean squares, and significance levels for disease index (DX), seed yield, maturity, plant height and lodging for two soybeans cultivars inoculated with *Fusarium solani* f. sp. g*lycines* using five irrigation treatments across 2002 and 2003.

		Mean Square				
Source of variation	df	DX	Yield	Maturity	Plant height	Lodging
Year	1	53.5 ^{ns}	1102894 ^{ns}	1814.5 *	2587.8*	6 ^{ns}
Rep(year)	6	45.7	174457	13.9	26.6	0.4
Irrigation	4	236.6 ^{ns}	2460143 ^{ns}	2.6 ^{ns}	18.1 ^{ns}	2.3 ^{ns}
Irrigation*Rep (Year)	24	36.5	77694	1.7	32.8	0.6
Year* Irrigation	4	75.7 ^{ns}	30374 ^{ns}	24.8 ^{ns}	57.5 ^{ns}	0.7 ^{ns}
Cultivar	1	1494.7 ^{ns}	1459121 ^{ns}	5.5 ^{ns}	20 ^{ns}	5.0 *
Cultivar*Year	1	57.1 ^{ns}	348692 ^{ns}	46.5 **	61.2 ^{ns}	0.1 ^{ns}
Cultivar* Irrigation	4	198.6 ^{ns}	275165 **	1.5 ^{ns}	38.2 ^{ns}	0.9 ^{ns}
Cultivar*year* Irrigation	4	67.9 ^{ns}	15505 ^{ns}	1.5 ^{ns}	17.3 ^{ns}	0.8 ^{ns}
Residual	30	38.2	1310.2	0.9	13.6	13.6

*, **, ***, ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 1.12. Means of disease index (DX), seed yield and maturity across two soybean cultivars receiving five irrigation treatments across 2002 and 2003.

Irrigation treatments	DX	Yield	Maturity
		kg ha^{-1}	days
Irrigation on V3, V7 and R4 (5)	12.1	4198	122.5
Irrigation on V3 and V7 (4)	8.9	4078	122.5
Irrigation on V3 (2)	5.7	4262	122.6
Irrigation on V7 (3)	4.3	4404	122.9
Natural rain (1)	2.4	4323	123.5
Mean	6.7	4253	122.8
LSD 5% †	8.5	579.6	1.4

† Least significant difference at P = 0.05.

Table 1.13. Means of disease index (DX), seed yield and maturity of two soybean cultivars receiving five irrigation treatments across 2002 and 2003.

	DX		Yield (kg ha^{-1})		Maturity (days)	
Irrigation treatments	AG3003	AG3302	AG3003	AG3302	AG3003	AG3302
Irrigation at V3, V7 and R4 (5)	22.5	1.7	3855	4541	122	123
Irrigation at V3 and V7 (4)	12.9	4.9	3916	4239	123	122
Irrigation at V3 (2)	4.3	2.4	4139	4331	124	122
Irrigation at V7 (3)	6.4	2.4	4410	4398	124	122
Natural rain (1)	4.3	0.5	4215	4431	124	122
Mean	11.0	2.3	4118	4388	123	122
LSD 5% †	9.8	9.8	574	574	1.4	1.4

† Least significant difference at P = 0.05.

Table 1.14. Sources of variation, degrees of freedom (df), mean squares, and significance levels for disease index (DX), seed yield, maturity, plant height and lodging for two soybeans cultivars inoculated with *Fusarium solani* f. sp. g*lycines,* using six irrigation treatments in 2004.

		Mean Square				
Source of variation	df	DX	Yield	Maturity	Plant height	Lodging
Irrigation	5	420.1 ^{ns}	374371^{ns}	7.2*	2.9 ^{ns}	0.4^{ns}
Rep	3	234.5	9288	1.8	4.2	0.7
Cultivar	1	811.2***	577881*	14.4**	3.2^{ns}	17.8***
Cultivar* Irrigation	5	43.7 ^{ns}	57743**	1.1^{ns}	6.1^{ns}	0.2^{ns}
Irrigation*Rep	14	161.1	371561	1.9	5.1	0.5
Residual	17	25.2	90333	1.6	2.2	0.9

*, **, ***, ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 1.15. Means of disease index (DX), yield and maturity of the cultivars AG3003 and AG3302 receiving six irrigation treatments in 2004.

	Disease Index (DX)		Yield (Kg ha^{-1})		Maturity (days)	
Irrigation treatments	AG3003	AG3302	AG3003	AG3302	AG3003	AG3302
Irrigation at V3, V7, R4 and R5 (5)	30.0	15.7	3364	3747	113.5	113.5
Irrigation at V3, V7 and R4 (4)	26.3	15.6	3445	3525	113.2	114.2
Irrigation at R4 (2)	22.6	12.2	3678	3897	113.7	115.2
Irrigation at R3, R4 and R5 (6)	17.6	7.8	3752	3870	115	116
Irrigation at V3 and V7 (3)	8.4	2.1	3895	4137	116	116
Natural rain (1)	4.3	4.9	3865	3840	116	117
Mean	18.2	10.4	3667	3836	114.2	115.7
LSD 5% †	16.2	16.2	728	728	2.3	2.3

† Least significant difference at P = 0.05.

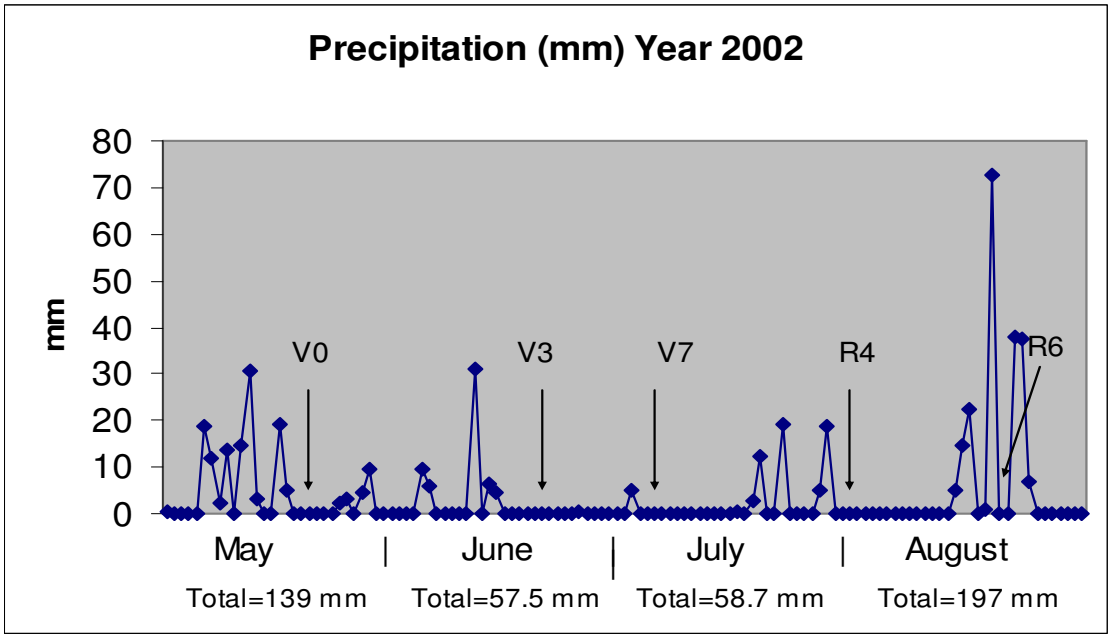

Figure 1.2. Precipitation (mm) from May to August, 2002. The growth stages when irrigation (75 mm) was applied are denoted by the arrows.

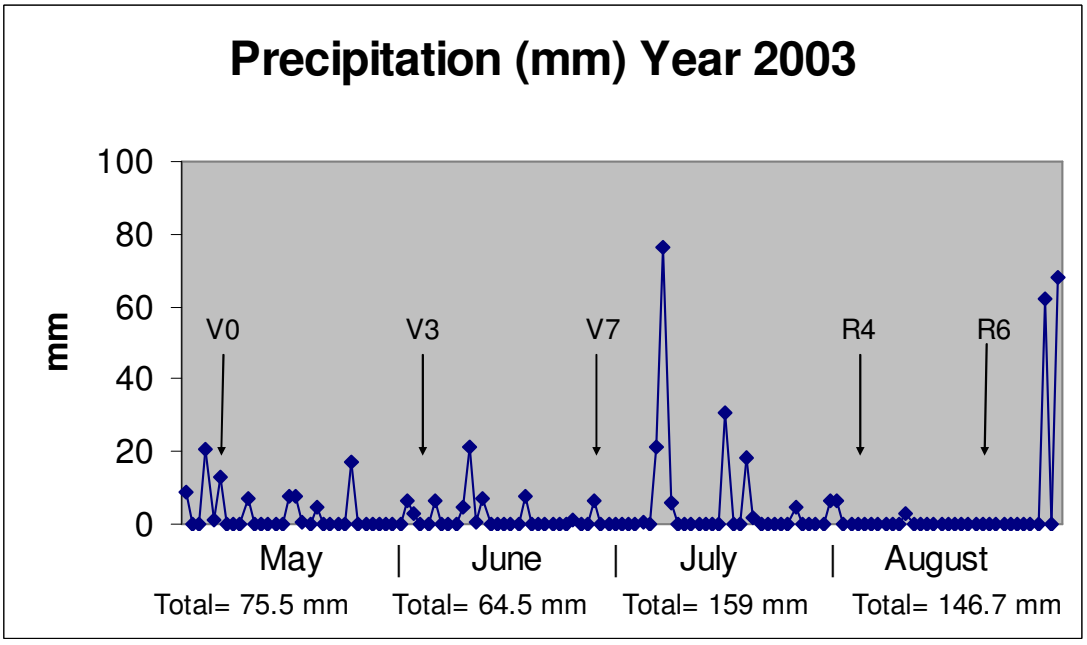

Figure 1.3. Precipitation (mm) from May to August, 2003. The growth stages when irrigation (75 mm) was applied are denoted by the arrows.

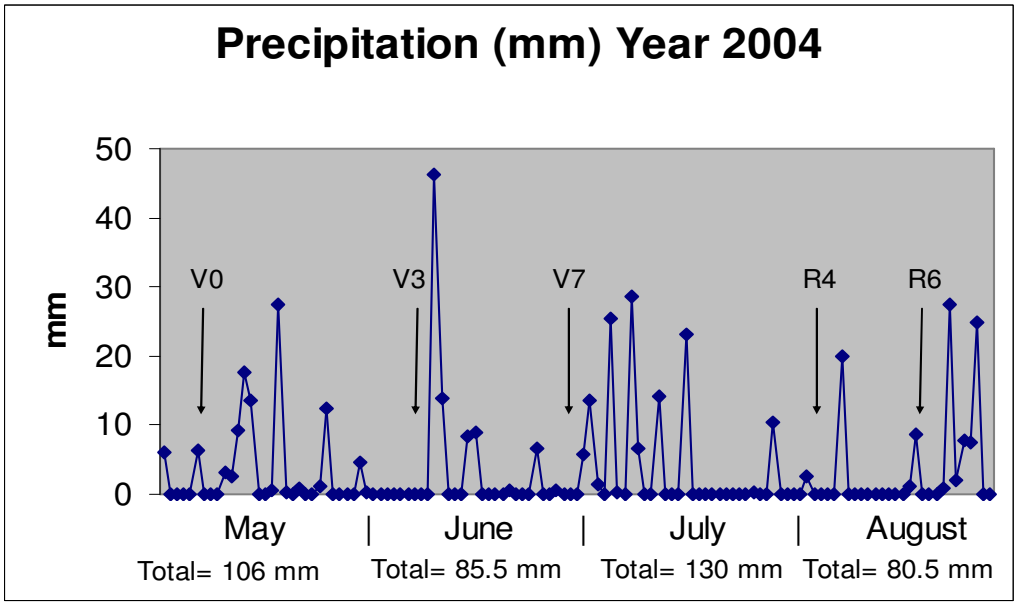

Figure 1.4. Precipitation (mm) from May to August, 2004. The growth stages when irrigation (75 mm) was applied are denoted by the arrows.

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CHAPTER TWO

EVALUATION OF TWO GREENHOUSE INOCULATION METHODS FOR SELECTION OF SOYBEAN [*Glycine max* (L.) Merr.] GENOTYPES FOR FIELD RESISTANCE TO SUDDEN DEATH SYNDROME

ABSTRACT

Sudden death syndrome (SDS) caused by the soilborne fungus *Fusarium solani* f. sp. g*lycines* (FSG) is a major disease in soybean [*Glycine max* (L.) Merr.]. Selection for SDS resistance in the field is difficult because of the impact of the environment on disease development. Evaluation of a great number of lines in the field is time consuming and expensive. The objective of this study was to evaluate two SDS greenhouse screening methods and determine which best correlates with field resistance of soybean genotypes. Three sets of genotypes were previously evaluated for field reaction to SDS and were placed into three classes (partially resistant, intermediate and susceptible) based on these reactions. All three sets were evaluated with the greenhouse cone method and two sets were evaluated with the greenhouse tray method. The plants were inoculated with FSG infested white sorghum [*Sorghum bicolor* (L.) Moench] grain and foliar symptom severity (disease severity, DS) was rated 21 days after emergence. The cone method separated the three classes in one set, but failed to separate the intermediate and susceptible classes in two sets of genotypes. The correlations between the SDS ratings of genotypes in the field and in the greenhouse with cones ranged from 0.58 ($p>0.001$) to 0.69 ($p>0.001$). The correlations of SDS ratings of genotypes between field and greenhouse tray ratings was 0.54 ($p>0.001$) for Set 1 and 0.39^{ns} for Set 2. We conclude that the cone method has

successfully predicted field results for all three sets evaluated, and hence can be used for predicting field SDS resistance reactions of genotypes. We concluded that cone method has successfully predicted field results for all three sets evaluated, and hence can be used for predicting field SDS resistance reactions of genotypes.

INTRODUCTION

Sudden death syndrome (SDS) is a soybean disease caused by the soilborne fungus *Fusarium solani* f. sp. *glycines* (FSG) (Gibson et al., 1994). The fungus infects plants through the roots and severely infected plants exhibit blackened and rotted taproots with few lateral roots (Stephens et al., 1993). Symptoms also include interveinal chlorosis and necrosis of leaves, vascular discoloration of stems, premature defoliation, and pod abortion (Rupe, 1989).

Screening for SDS reactions of soybean genotypes has been done under field conditions (Hartwig et al., 1996; Schmidt et al., 1999) and in the greenhouse (Hartman et al., 1997; Mueller, 2001). Selection for SDS resistance in the field is difficult because the occurrence of the disease is unpredictable due to the sensitivity of symptom development to environmental factors (Gibson et al., 1994; Nijti et al., 1996). In addition, the evaluation of a great number of lines in the field is time consuming and expensive. Researchers have evaluated SDS resistance in the greenhouse by inoculating plants with FSG infested oat (Lim and Jim, 1991; Stephens et al., 1993), sorghum (Hartman et al., 1997; Mueller, 2001), cornmeal (Killebrew et al., 1998) or tootpicks (Klingesfuss et al., 2002).

Stephens et al. (1993) evaluated the reaction of 12 soybean cultivars grown in soil inoculated with FSG infested oats in the field and in pots in a greenhouse. The correlation between field and greenhouse SDS ratings of cultivars ranged from 0.60 to 0.90. However,

according to Torto et al. (1996), researchers may better predict field responses of genotypes to SDS with greenhouse assays by reducing greenhouse inoculation rates. Inoculations in the greenhouse typically have greater than 10,000 spores cm^{-3} of plant growth medium compared to infested field rates of typically less than 5,000 spores cm^{-3} of plant soil (Torto et al., 1996). Nijti et al. (2001) compared selection for field resistance to SDS in the greenhouse at varying inoculum rates. For that, spores were counted and these spore counts were used to calculate the volume of culture necessary for each inoculum rate. The moderate inoculum rate of 4,000 spores cm^{-3} of plant growth medium resulted in the best correlation (0.77) with the field results.

The development of a simple and reliable greenhouse screening method is important in the selection of soybean plants for resistance to SDS. The objective of this study was to compare two greenhouse inoculation methods to determine which best correlates with the field reaction of soybean genotypes to SDS.

MATERIAL AND METHODS

Plant material

Three sets of genotypes were used in this study. Set 1 included 30 recombinant inbred lines (RILs) selected from a population of 100 lines derived from the cross of the SDS susceptible cultivar Essex (Smith and Campes, 1973) with the SDS partially resistant cultivar Forrest (Hartwig and Epps, 1973). The RILs were previously selected for SDS leaf scores across five field environments and were characterized into the following three resistance classes: (i) partially resistant (PR), the 10 most resistant of the 100 lines, eight of which were significantly more resistant than Forrest; (ii) intermediate (IN), the 10 lines with resistance ratings closest to population mean; (iii) SDS susceptible (S), the 10 least resistant lines, all of which were

significantly more susceptible than Essex (Hnetkovsky et al., 1996). The field plots were rated at the R6 growth stage (Fehr and Calviness, 1971) for disease incidence (DI) and disease severity (DS) and the disease index (DX) was calculated according to Gibson (1994). Set 2 included 24 soybean cultivars and lines with characterized field resistance to SDS. These genotypes were previously evaluated for SDS disease incidence and severity in at least three field environments and classified as partially resistant, intermediate and susceptible. The genotypic scores were based on the average percentage of disease incidence relative to the susceptible control in each experiment. Set 3 included 92 RILs derived from the cross of the SDS partially resistant cultivar 'Ina' (Nickel et al., 1998) with the SDS susceptible experimental line LN 91-1695. The RILs were evaluated in the field for disease incidence (DI) and disease severity (DX) in Urbana, IL over the years of 2003 and 2004. The DI (0-100%) and DS (1-9) were taken according to Gibson et al. (1994) at the R6 growth stage. Disease incidence was taken as the percentage of plants with foliar symptoms. Foliar DS was recorded as: 1= 0 to 10% chlorosis or 1 to 5% necrosis, 2 = 10 to 20% chlorosis or 6 to 10% necrosis, 3 = 20 to 40% chlorosis or 10 to 20% necrosis, 4 = 40 to 60% chlorosis or 20 to 40% necrosis, 5 = > than 60% chlorosis or > than 40% necrosis, 6 = up to 33% defoliation, 7 = up to 66% defoliation, 8 = > than 66% defoliation and 9 = premature death of the plant. A disease index (DX; 0-100) was calculated as (DI x DS)/9. Based on the DX field ratings of the RILs, these were grouped into three classes: (i) partially resistant (PR), the 10 most resistant RILs, (ii) intermediate (IN), the 10 lines with resistance reactions closest to the population mean and (iii) susceptible (S) the 10 more susceptible RILs.

Inoculum production

The Monticello isolate of FSG (Hartman et al., 1995) was increased on white sorghum seed that were soaked overnight and autoclaved twice in 1-liter flasks. Each flask with 300 g of

sorghum seeds was inoculated with ten, 4 mm diameter plugs of fungal mycelium and incubated for 2 weeks. The colony forming units (CFU) of the infested sorghum inoculum was determined as previously described on hairy roots (Li et al., 2002) with slight modification. Briefly, 1 gram of sorghum inoculum was soaked in a 250-ml Erlenmeyer flask containing 100 ml of sterile distilled water. The flasks were shaken at 150 rpm on an orbital shaker for 30 min, and then serially diluted 10 fold with sterile distilled water two times. From each dilution, 100 µl of inoculum dilution was spread on an agar plate (100 x 15 mm) containing *Fusarium solani* f. sp. *glycines* semi-selective medium (Huang and Hartman, 1996). Six plates were used for each inoculum dilution. The plates were incubated at room temperature (25 ± 2ºC) for 10 days. Colonies of *Fusarium solani* f. sp. *glycines* were identified as described previously (Li et al., 2000). The number of colonies on each plate was counted and used to calculate the number of colony-forming units per gram of sorghum. The experiment was run two times.

Cone method

One hundred ml of steam-treated soil mix (2:1 sand:soil) was placed in a SC-10 type cone and 5 ml (3 g) of FSG infested white sorghum seeds were placed on the top of the soil mix. Twenty ml of soil mix were added to cover the infested seeds and three seeds were added to each cone, which were covered with another 20 ml of soil mix.

After seedling emergence, seedlings were thinned leaving one seedling per cone. The soil was maintained to near the water-holding capacity by flooding the cones twice a day. The cone was the experimental unit and these were arranged in a randomized complete block design (RCBD) with four replicates. The plants were rated 21 days after germination using a scale from 1 (no symptoms) to 6 (severe symptoms) based on leaf chlorosis and necrosis, defoliation, and premature plant death. The rating scale was the following: 1 = no symptoms, 2 = slight symptom

development, with chlorosis on leaves (1 to 20% foliage affected), 3 = moderate symptom development, with chlorosis and necrosis on leaves (20 to 40% foliage affected), 4 = heavy symptom development, with chlorosis and necrosis on leaves (40 to 60% foliage affected), 5 = severe symptom development, with chlorosis and necrosis on leaves (60 to 80% foliage affected), 6 = severe symptom development, with with chlorosis and necrosis on leaves (more than 80% foliage affected). For set 1 and 3, the experiments were done twice one month apart. Hence, there were a total of eight replicates per genotype for Set 1 and 3. For Set 2, the experiment was done once and five replicates were used.

Tray method

Greenhouse steam-treated soil mix (2:1 sand:soil) was placed in 37 x 52 cm galvanized trays to a depth of 4 cm. A template was used to make 7 furrows that were 36 cm long, 2 cm deep, and 7 cm apart, and 10 ml of infested sorghum seeds was evenly distributed in each furrow. Soil mix was added to cover the infested seeds to a depth of 2 cm. The template was reapplied to make a 2-cm deep furrow directly over the inoculum. Three experimental units (each was a 12 cm long row) were placed per furrow resulting in each tray holding 21 experimental units. Five seeds per entry were sown in each experimental unit, which was covered with soil mix to a depth of 2 cm.

The soil was maintained near the water-holding capacity by flooding the trays twice daily. The 12 cm furrow was the experimental unit and the experimental design was a RCBD with four replicates for Set 1 and three replicates for Set 2. The plants were rated for SDS symptoms 21 days after germination using the scale previously described. The DS score was based on the mean of the plants per experimental unit.

Set 1 and 2 were evaluated independently for foliar resistance to SDS in the greenhouse using both the cone and tray methods. Non-inoculated controls were included in the experiments. Set 3 was evaluated only with the cone method. The experiments were conducted with a 12 hour photoperiod and with air temperature of 25º ± 2 ºC in the greenhouse. The experiments were conducted during the winter of 2002 and 2003.

Statistical analysis

An analysis of variance was computed for the greenhouse data using PROC MIXED (SAS Institute, 2000). All factors were considered fixed except for blocks. Means were separated using least significance differences (LSD) at 5%. Normality and homogeneity of variances for the data were verified. The CORR PROCEDURE of SAS was used to calculate Pearson correlations between field DX and greenhouse DS data (DS correlation) and between field genotypic rankings and greenhouse genotypic rankings (ranking correlation). In each of the three sets, genotypes were classified into two classes, partially resistant (PR) and susceptible (S) according to the DS scores observed in the greenhouse tests. This classification was based on the controls and the LSD (5%) of each experiment. The genotypes with a DS score similar to the susceptible check ± LSD (5%) were classified as susceptible and the genotypes with a DS score similar to the partially resistant check ± LSD (5%) were classified as resistant.

RESULTS AND DISCUSSION

Genotypes of the three sets grown in the greenhouse in soil inoculated with infested sorghum showed typical SDS foliar symptoms for both the cone and tray methods. The noninoculated control plots didn't show any SDS foliar symptoms. The CFU of the infested sorghum used in the experiments averaged 2.4 x 10^5/g inoculum.

Cone method

Analysis of variance across experiments for Sets 1 to 3 (Tables 2.1 and 2.2) showed significant effects for reaction to SDS with the cone method among resistance classes that were defined based on field results. Contrasts between classes (Tables 2.3 and 2.4) showed that the cone method was able to separate the means of all three classes from each other for Set 1 genotypes. For Sets 2 and 3, the IN class did not differ significantly from the S class. Set 2 is composed of cultivars and lines from different maturity groups and these were evaluated in different field experiments. The ranking of these genotypes might not be very accurate and, in addition, genotypes from different backgrounds might have different responses to greenhouse inoculation compared to field reactions to the disease. This could have made the separation of classes IN and S difficult.

No significant differences among genotypes within classes were observed for Sets 1 and 3 (Table 2.1). The lines from the same classes were selected based on similarity for SDS field response in Sets 1 (Njiti et al., 2001) and 3. Conversely, significant differences among genotypes within classes were observed for Set 2.

Correlations between the field DX or DI scores and the greenhouse cone DS scores were significant for the three sets (Tables 2.5, 2.6 and 2.7). The greatest correlation was 0.69 ($p>0.001$), observed for Set 1, while the lowest was 0.59 ($p>0.001$) for Set 2. When all 94 lines from the cross Ina by LN 91-1695 were included in the correlation analysis, the DS correlation was 0.50 ($p>0.001$). Even greater correlations were observed for the rank correlations. The values found were 0.74 ($p>0.001$) for Set 1, 0.61 ($p>0.001$) for Set 2 and 0.65 ($p>0.001$) for Set 3. A ranking correlation of 0.50 was detected when all 94 lines from the cross Ina by LN 91-1695 were included.

The good association between field and greenhouse cone evaluations can be seen through the means of field DX or DI scores and cone DS scores and the ranking of genotypes in Set 1 (Table 2.8), Set 2 (Table 2.9), and Set 3 (Table 2.10). Most genotypes had the same resistance classification for both the greenhouse cone and field evaluations.

Tray method

Analysis of variance of greenhouse tray DS scores showed significant effects among field based resistance classes for Set 1 and non significant effects for Set 2 (Table 2.2). This method could only separate the PR from the S class on Set 1, although the significance levels of the contrasts IN vs PR and IN vs S were very close to 5%. For Set 2, the DS averages of the three classes were similar (Table 2.11).

No differences among genotypes within classes were observed for Set 1, while highly significant differences were found for Set 2 (Tables 2.2 and 2.4). Correlations between field and tray scores were lower than the correlations between field and cone scores. For Set 1 the Peason correlation between field DX and tray DS was 0.54 ($p>0.001$) and the rank correlation was 0.56 ($p>0.001$). For Set 2 no significant correlations were found between field and tray resistance scores (Tables 2.5 and 2.6).

It is not clear why better correlations with field results were observed with the cone method than the tray method. For both methods, the same inoculum, greenhouse, planting depth, and amount of water were used. One possible reason for the greater correlations with the cone method was that roots are less likely to escape the infested grain with this method than the tray method. This is because a continuous layer of infested grain is placed in the cones, whereas for the tray method, infested grain is only placed under to seed. This allows the roots to potentially grow around the inoculum with the tray method, thus escaping the disease. This disease escape

can be observed with the tray method in Set 2, in which a reasonable number of field susceptible lines were classified as partially resistant. However, some field partially resistant lines were classified as susceptible, showing that the inoculum overcame the resistance of these lines as observed by Njiti et al. (2001).

Soil temperature is a variable that is not controlled in our SDS greenhouse assays. By controlling soil temperature, we may be able to improve the consistency of our results within and across experiments. In addition, an optimal soil temperature may be found that improves the greenhouse correlation with field results. A water bath system that controls soil temperature is used in screening soybean genotypes for SCN resistance (Niblack et al., 2002) and this system could be adapted to SDS resistance screening.

In summary we conclude that the cone method has successfully predicted field results for all three genotype sets evaluated. Thus, this method can be used for screening genotypes for reaction to SDS in greenhouse. The tray method showed good results for Set 1, but could not predict field results for the Set 2 of genotypes.

Table 2.1. Sources of variation, degrees of freedom (df), mean squares, and significance values for sudden death syndrome disease severity (DS) for two sets of soybean genotypes using cones inoculated with *Fusarium solani* f. sp. g*lycines* in the greenhouse.

Source of variation	Set 1		Set 3	
	df	DS	df	DS
Experiment	1	4.8268 ^{ns}	1	4.9193 ^{ns}
Class	2	22.7965***	2	12.7090***
Class x Experiment	2	1.4833 ^{ns}	2	0.9720 ^{ns}
Block (experiment)	8	2.0020 *	8	2.2966 *
Genotype (class)	27	1.8256 ^{ns}	27	1.5979 ^{ns}
Genotype x experiment (class)	27	3.1659***	27	1.6377 *
Residual	171	1.2994	171	1.0530

*, **, ***, ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 2.2. Sources of variation, degrees of freedom (df), mean squares, and significance values for sudden death syndrome disease severity (DS) for Set 2 genotypes inoculated with *Fusarium solani* f. sp. g*lycines* using the greenhouse cone and tray methods.

	Cone method		Tray method			
	Set 2		Set 1		Set 2	
Source of variation	df	DS	df	DS	df	DS
Class	2	6.817287**	2	4.921892***	2	0.1767 ns
Block	4	0.381510ns	3	3.411622*	2	0.2019***
Genotype (class)	21	1.325710**	27	0.666611 ns	21	0.1423***
Residual	92	0.511402	85	0.539394	41	0.1263

*, **, ***, ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 2.3. Estimates and levels of significance from contrasts among classes of genotypes partially resistant (PR), intermediate (IN) and susceptible (S) to sudden death syndrome using the greenhouse cone method inoculated with *Fusarium solani* f. sp. g*lycines*.

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	Set 1		Set 2		Set 3	
Classes	Estimate	Pr > |t|	Estimate	Pr > |t|	Estimate	Pr > |t|
IN vs PR	0.4778	0.0346	0.6493	0.0169	0.6200	0.0062
IN vs S	-0.6108	0.0084	-0.0919	0.7335	-0.2774	0.1904
PR vs S	-1.0886	<.0001	-0.7413	0.0094	-0.8974	0.0002

Table 2.4. Sudden death syndrome (SDS) disease severity means for the greenhouse cone and tray inoculation methods for genotypes in Sets 1, 2 and 3. The genotypes were placed into classes based on field SDS ratings.

Field SDS class	Set 1	Set 2	Set 3
	----------Disease severity----------		
Cone method			
Partially resistant	2.5	2.5	2.5
Intermediate resistant	3.0	3.1	3.2
Susceptible	3.6	3.2	3.5
LSD 5% †	0.44	0.54	0.43
Tray method			
Partially resistant	2.4	3.5	
Intermediate resistant	2.8	3.3	
Susceptible	3.1	3.6	
LSD 5% †	0.38	0.24	

† Least significant difference at P = 0.05.

Table 2.5. Correlation coefficients between field sudden death syndrome disease index (DX) and disease severity (DS) in greenhouse cone and tray inoculations for Set 1 genotypes based on genotypic means and rankings.

	Mean field DX	Mean cone DS	Mean tray DS	Ranking field DX	Ranking cone DS	Ranking tray DS
Mean field DX		0.69***	0.54***			
Mean cone DS	0.69***		0.48***			
Mean tray DS	0.54***	0.48***				
Ranking field DX					0.74***	0.54**
Ranking cone DS				0.74***		0.56***
Ranking tray DS				0.54**	0.56***	

*, **, ***, ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 2.6. Correlation coefficients between field sudden death syndrome disease incidence (DI) and disease severity (DS) in greenhouse cone and tray inoculations for Set 2 genotypes based on means and rankings.

	Mean field DI	Mean cone DS	Mean tray DS	Ranking field DI	Ranking cones DS	Ranking trays DS
Mean field DI		0.59^{***}	0.38^{ns}			
Mean cone DS	0.59^{***}		0.29^{ns}			
Mean tray DS	0.38^{ns}	0.29^{ns}				
Ranking field DI					0.61^{***}	0.35^{ns}
Ranking cone DS				0.61^{***}		0.38^{ns}
Ranking tray DS				0.35^{ns}	0.38^{ns}	

*, **, ***, ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 2.7. Correlation coefficients between field sudden death syndrome disease index (DX) and disease severity (DS) in greenhouse cone and tray inoculations for Set 3 genotypes based on genotypic means and rankings.

	Mean field DX	Mean cone DS	Ranking field DX	Ranking cone DS
[+]Mean field DX		0.51***		
[+]Mean cone DS	0.51***			
[+]Ranking field DX				0.50***
[+]Ranking cone DS			0.50***	
[++]Field DX		0.68***		
[++]Cone DS	0.68***			
[++]Ranking field DX				0.65***
[++]Ranking cone DS			0.65***	

*, **, ***, ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

[+]Includes all 94 genotypes of the set.

[++] Includes only 30 genotypes of the three SDS classes.

Table 2.8. Means and rankings of soybean lines for field disease index (DX) and disease severity (DS) for cone and tray inoculation methods for Set 1 genotypes.

Genotype	Field DX	Ranking Field DX	Cone DS	Cone class	Ranking Cones DS	Tray DS	Tray class	Ranking tray DS
Field partially resistant class								
Ls-G96	0.1	1	2.6	PR	8	2.6	PR	13
(E x F) 23	0.5	2	2.1	PR	5	3.3	S	24
(E x F) 59	0.5	3	2.7	PR	9	2.2	PR	6
(E x F) 67	1.1	4	2.9	PR	13	2.1	PR	3
(E x F) 57	1.1	5	2.6	PR	7	2.0	PR	1
(E x F) 44	1.1	6	1.7	PR	2	2.1	PR	2
(E x F) 20	1.2	7	2.8	PR	10	2.6	PR	12
(E x F) 47	1.5	8	2.0	PR	3	2.5	PR	8
(E x F) 37	1.9	9	3.3	PR	21	3.0	PR/S	20
(E x F) 55	3.0	10	2.1	PR	4	2.1	PR	4
Forrest			2.2	PR		2.1	PR	
Field SDS intermediate resistant class								
(E x F) 46	5.4	11	1.7	PR	1	2.4	PR	7
(E x F) 14	6.3	12	3.1	PR	15	2.2	PR	5
(E x F) 91	6.4	13	3.1	PR	18	2.6	PR	11
(E x F) 75	6.4	14	4.0	S	28	3.1	PR/S	22
(E x F) 49	6.9	15	2.3	PR	6	3.5	S	27
(E x F) 26	7.2	16	3.6	S	23	3.2	S	23
(E x F) 6	8.2	17	3.1	S	17	2.9	PR/S	18
(E x F) 73	8.6	18	2.9	PR	12	2.7	PR	16
(E x F) 97	9.1	19	3.1	PR	16	2.6	PR	9
(E x F) 45	9.7	20	2.9	PR	11	2.7	PR	15
Field SDS susceptible class								
(E x F) 39	15.7	21	3.6	S	25	3.6	S	28
(E x F) 51	16.0	22	3.3	PR	20	2.9	PR/S	19
(E x F) 10	17.5	23	3.0	PR	14	3.4	S	26
(E x F) 68	18.2	24	3.2	PR	19	3.3	S	25
(E x F) 18	18.6	25	3.3	PR	22	2.6	PR	14
(E x F) 83	18.7	26	3.8	S	26	3.7	S	30

Table 2.8. (continued)

(E x F) 76	18.7	27	3.6	S	24	2.6	PR	10
(E x F) 85	19.8	28	4.0	S	29	3.6	S	29
(E x F) 80	20.2	29	3.8	S	27	3.0	PR/S	21
(E x F) 7	20.3	30	4.0	S	30	2.7	PR	17
Essex			4.7	S		3.9	S	
Mean			3.0			2.8		
LSD 5%			1.21			1.02		

Table 2.9. Means and rankings of soybean lines for field disease index (DX) and disease severity (DS) for cone and tray inoculation methods for Set 2 genotypes.

Genotype	Field DI [+]	Field Ranking	Cone DS	Ranking Cone DS	Cone Class[‡]	Tray DS	Ranking Tray DS	Tray class[‡]
Field SDS partially resistant class								
A5560	0	1	1.8	2	PR	3.3	18	PR/S
LS90-1920	2	2	2.9	12	S	3.2	13	PR/S
LS94-3207	3	3	2.7	9	PR	2.8	3	PR/S
Manokin	5	4	2.5	6	PR	3	5	PR/S
PI 520733	5	4	2.3	4	PR	2.7	1	PR/S
Ripley	6	6	3.2	15	S	3.1	13	PR/S
Pharaoh	8	7	2.6	8	PR	3	5	PR/S
Cordell	10	8	1.8	3	PR	3	5	PR/S
Jack	12	9	2.5	5	PR	3	5	PR/S
Forrest	16	10	1.7	1	PR	3.2	13	PR/S
LS93-0375	20	11	3.6	22	S	3.3	18	PR/S
PI567374			2.0		PR	2.6		PR
Field SDS intermediate resistant class								
Egyptian	32	12	2.7	10	PR	3	5	PR/S
Essex	39	13	3.4	18	S	3	5	PR/S
Calland	43	14	3.6	23	S	3	5	PR/S
Pella86	44	15	3.2	14	S	2.7	1	PR/S
A4715	54	16	3.5	20	S	3.3	18	PR/S
A5403	57	17	3.3	16	S	3.3	18	PR/S
Field SDS susceptible class								
Hutcheson	69	18	2.6	7	PR	3.2	13	PR/S
Douglas	75	19	2.8	11	PR/S	2.8	2	PR/S
DP105	100	20	3.6	21	S	3.2	13	PR/S
Spencer	100	20	3.6	24	S	3.3	18	PR/S
P3981	100	20	3.2	13	S	3	5	PR/S
CM497	100	20	3.4	19	S	3.7	24	PR/S
V82-2191	100	20	3.3	17	S	3.3	18	PR/S
Spencer			3.7		S	3.3		S
Mean			2.9			3.1		
LSD 5%			0.90			0.62		

[+] Percentage disease incidence relative to susceptible check of each experiment

Table 2.10. Means and rankings of soybean lines for field disease index (DX) and disease severity (DS) for cone inoculation method for Set 3 genotypes.

Genotype	Field DX	Ranking Field DX	Cone DS	Ranking cone	Class
Field SDS partially resistant class					
96	0	1	2.2	4	PR
33	0	2	2.9	13	PR
53	0.1	3	2.6	7	PR
44	0.1	4	3.6	26	S
46	0.1	5	2.6	6	PR
16	0.2	6	2.2	3	PR
40	0.2	7	2.1	1	PR
13	0.4	8	2.1	2	PR
35	0.4	9	2.7	10	PR
15	0.4	10	2.3	5	PR
Ina	0.3		2.1		PR
Field SDS intermediate resistant class					
81	4	11	2.8	12	S
45	4	12	3.2	20	PR
10	4.1	13	3.4	21	S
77	4.4	14	2.7	8	S
25	4.5	15	3	16	S
69	4.5	16	3.2	19	PR
54	4.7	17	3	15	PR
47	4.9	18	2.7	11	PR
41	4.9	19	3.5	25	S
14	4.9	20	3	17	PR
Field SDS susceptible class					
66	8.1	21	3.9	29	S
98	9.1	22	3.9	30	S
21	9.4	23	2.7	9	PR
63	10.5	24	3.4	22	S
11	11.0	25	2.9	14	PR
71	11.4	26	3.2	18	PR
19	11.7	27	3.5	23	S
88	13.5	28	3.5	24	S
50	16.3	29	3.6	27	S
29	19.2	30	3.7	28	S
LN91-1695	11.9		4.5		S
Mean	5.6		3.1		
LSD 5%	4.0		1.097		

Table 2.11. Estimates and levels of significance from contrasts among genotypes classified in the field as partially resistant (PR), intermediate resistant (IN) and susceptible (S) to sudden death syndrome (SDS) using greenhouse tray method inoculated with *Fusarium solani* f. sp. g*lycines*

	Set 1		Set 2	
classes	Estimate	Pr > |t|	Estimate	Pr > |t|
IN vs PR	0.3671	0.0501	0.0086	0.9418
IN vs S	0.3533	0.0653	-0.1255	0.3288
PR vs S	-0.7204	0.0006	-0.1077	0.2270

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CHAPTER THREE

QTL MAPPING OF RESISTANCE TO *Fusarium solani* f. sp. g*lycines* IN SOYBEAN [*Glycine max* (L.) Merr.]

ABSTRACT

The use of resistant cultivars is the most effective method for controlling sudden death syndrome (SDS). Six quantitative trait loci (QTL) associated with SDS resistance have been reported. The objective of my study was to detect QTL conferring SDS resistance in two populations of recombinant inbred lines (RILs). Ninety six RILs from the cross PI 567374 x Omaha and 92 RILs from the cross Ina x LN91-1695 were evaluated for SDS seedling resistance in the greenhouse. The Ina x LN91-1695 RILs also were evaluated for SDS resistance in the field. Simple sequence repeats (SSR) markers were used to map QTL in both populations. For the PI 567374 x Omaha population, two chromosomal regions were associated with seedling SDS resistance at P< 0.005. The first significant QTL was mapped with Satt311 (P = 0.0032, R^2 = 12%) onto LG D2. The second significant QTL was mapped with Sat_299 (P = 0.0009, R^2 = 12%) onto LG I. The beneficial alleles for both regions were from the resistant parent PI 567374. Five chromosomal regions were associated with field SDS resistance in the Ina x LN91-1695 population. Two QTL mapped to the same regions as SDS resistance alleles were previously found in the cultivar Essex. These were mapped by Satt270 (P = 0.0028, R^2 = 12.7%) onto LG I and by Satt371 (P= 0.02 R^2 = 12%) onto LG C2. The beneficial alleles were from the resistant parent Ina for both QTL. A second region on LG I was mapped by Sct_189 (P= 0.0139, R^2 = 9.8%) and the beneficial allele was from the susceptible parent LN91-1695. Two additional regions were mapped onto LG A1 by Satt684 (P= 0.02 R^2 = 8.5%) and onto LG E by Satt268 (P

= 0.01, R^2 = 9.8%) and their beneficial alleles were from Ina. Three out of five QTL mapped with the field data were also mapped with the greenhouse data.

INTRODUCTION

Sudden death syndrome (SDS) is a mid to late season fungal disease caused by *Fusarium solani* f. sp. g*lycines* (FSG) that can cause severe losses to soybean [*Glycine max* (L.) Merr.] yield (Gibson et al., 1994). First documented in the US in 1971 in Arkansas, the disease now occurs in many states (Roy, 1997).

The fungus is soilborne and infects plants through the roots resulting in a reduction of both root mass and number of viable root nodules (Rupe, 1989; Roy et al., 1989). The above ground symptoms include interveinal chlorosis and necrosis of leaves, premature defoliation, and pod abortion (Hartman et al., 1997). The leaf symptoms are proposed to be caused by fungal toxins (Li et al., 2000). Reductions in yield, ranging from slight to nearly 100 % (Hartman et al., 1999), result from SDS infection.

There are some practices that can reduce SDS occurrence such as subsoiling compacted fields (Vick et al., 2003), delaying planting and planting early cultivars (Hershman, 1996). However, the use of resistant cultivars is the most effective method for controlling SDS. Some cultivars and lines with good levels of resistance have been identified (Hartman et al., 1997; Hartwig et al., 1996; Schmidt et al., 1999; Mueller, 2001). However, Mueller (2001) reported that among 1670 cultivars evaluated, only 2 % were classified as partially resistant, emphasizing the need for more resistance in cultivars. In greenhouse tests, monogenic resistance to leaf scorch has been reported in the cultivar Ripley (Stephens et al., 1993). Under field conditions, resistance to SDS is described as polygenic (Hnetkovsky et al., 1996; Chang et al., 1996) and conditioned

by a minimum of five loci (Meksen et al., 1999; Njiti et al., 1996). This resistance to SDS was partial (Nijti et al., 1996; Igbal et al., 2001) since all soybean genotypes tested have shown some SDS symptoms under severe disease pressure. The field resistance was reported to include both leaf scorch resistance loci (Gibson et al., 1994) and root resistance loci (Njiti et al., 1997; Prabhu et al., 1999)

Due to the quantitative nature of the trait and the interactions between resistance loci and the environment, effective selection for field resistance requires multiple environments (Nijti et al., 2001). The high cost and long time required for evaluating SDS resistance in the field supports the use of marker-assisted selection as a valuable selection tool for plant breeders in the development of SDS resistant cultivars. Hnetkovsky et al. (1996) working with recombinant inbred lines (RIL) from a cross between the SDS partially resistant cultivar Forrest (Hartwig and Epps, 1973), and SDS susceptible cultivar Essex (Smith and Camper, 1973), identified two quantitative trait loci (QTL) for SDS resistance located on linkage group (LG) G. For both QTL, the resistance allele was derived from the cultivar Forrest, and the QTL jointly accounted for 34 % of the total phenotypic variability of disease incidence (DI). Later, Iqbal et al. (2001), working with the same population, defined two more resistant QTL alleles derived from Forrest. The four QTL jointly explained 50 % of the variation for DI. Additionally, the authors defined two more QTL on LG I and LG C2, which derived their resistance alleles from Essex. These two QTL jointly explained 40 % of the variation for SDS DI. All six loci together explained 91% of the variation in SDS DI in the population. The authors suggested that cultivars with durable resistance to SDS can be developed via gene pyramiding.

Nijti et al. (2001), working with RILs from a cross between the partially resistant cultivar Pyramid (Myers and Schimdt, 1988) and SDS susceptible cultivar Douglas (Nickel at al., 1982),

identified SDS resistance QTL that derived their beneficial alleles from Pyramid. These alleles were located on LG G where SDS resistance QTL were previously identified in Forrest. A third QTL, with the resistance allele derived from Douglas, was mapped to LG C2 where a SDS resistance QTL allele was previously mapped from Essex. More recently, a SDS resistance QTL located on LG D2 was mapped in a population developed from a cross between the cultivars Ripley (Cooper et al., 1990) and Spencer (Wilcox et al., 1989). The resistance allele for this QTL was derived from Ripley (Brian Diers, personal communication).

Additional research is needed to explore the nature of resistance of SDS, identify new sources of resistance, establish new and better ways to evaluate resistance and incorporate this resistance into elite cultivars (Roy, 1997; Muller, 2001). The identification of new loci with distinct mechanisms of resistance plays an important role for using gene pyramiding as an effective method for developing cultivars with stable SDS resistance (Nijti et al., 1998; Nijti et al., 2001). In this regard, the use of plant introductions (PIs) may be important, since PIs have been successfully used as sources of resistance genes to other diseases such as phytophthora (*Phytophthora sojae* Kaufmann and Gerdermann) soybean cyst nematode (*Heterodora glycines* Ichinoche) and brown stem rot (*Phialophora gregata* f.sp. *sojae*) (Carter et al., 2004).

The objectives of this research were (i) to evaluate the SDS resistance of soybean lines developed from a cross between the SDS partially resistant plant introduction PI 567374 and the SDS susceptible cultivar Omaha (Nickel at al., 1998) and from a cross between the SDS partially resistant cultivar Ina (Nickel et al., 1999) and the SDS susceptible experimental line LN 91-1695 and (ii) to detect QTL conferring SDS resistance in both populations.

MATERIAL AND METHODS

Plant material

The plant material used in this study were: (i) 96 F_4-derived RILs from a cross between the SDS partially resistant plant introduction PI 567374 and the SDS susceptible cultivar Omaha and (ii) 92 F_4-derived RILs from a cross between the SDS partially resistant cultivar Ina and the SDS susceptible experimental line LN 91-1695.

PI 567374 is a maturity group IV accession acquired from Shaanxi province in central China. The PI showed a high level of SDS foliar resistance (Hartman et al. 1997). Ina was developed by the Illinois Agricultural Experiment Station at the University of Illinois and released in 1998. This cultivar showed a high level of resistance to SDS both in the field (Brian Diers, personal communication) and greenhouse (Farias Neto et al., 2003). Ina is classified as a maturity group IV cultivar and it originated from the cross 'Jack' (Nickell et al., 1990) x 'Hartwig' (Anand, 1992). Hartwig is a SDS partially resistance cultivar (Anand, 1992) and it likely derives its SDS resistance alleles from Forrest. Jack also shows a good level of SDS resistance (Hartman et al., 1997, Nijti et al., 1997) and its source of SDS resistance is unknown.

Field experiments

The RILs developed from PI 567374 by Omaha cross, along with the parents and controls, were evaluated in the field for SDS resistance during 2002 and 2003. The RILs from Ina by LN 91-1695 cross, along with the parents and controls, were evaluated in the field for SDS resistance during 2002, 2003 and 2004. The experiments were conducted in Urbana, IL and were sown on May 16 in 2002, on April 28 in 2003, and on April 29 in 2004. The experiments

followed a randomized complete block design (RCBD) with three replicates each year. The experimental unit was a 2-row plot with a length of 3.5 meters and a row spacing of 75 cm. The seeding rate was 350,000 seeds ha^{-1}.

The plots were inoculated each year with FSG infested white sorghum [*Sorghum bicolor* (L.) Moench] grain placed below the seed prior to planting. The infested sorghum seeds (70 kg ha^{-1}) were planted with a plot planter at a depth of 8 cm. The planter was then reset to a 3 cm depth and the soybean seeds were planted directly over the sorghum. In addition to natural rain, the nurseries were irrigated three times, at V3, V7 and R3-R4 stages (Fehr and Calviness, 1971), to provide sufficient soil moisture to favor the occurrence of the disease. Irrigation was applied with a trickle irrigation system. The irrigation tapes were placed next to the two rows of each plot and the equivalent to 76 mm of rain was applied to the plots at each irrigation application.

Collection of field data

The RILs were rated for maturity date and SDS foliar symptoms. Maturity date was taken as the date when 95% of the pods had turned to their mature color (Fehr and Calviness, 1971). Disease incidence (DI) and disease severity (DS) were rated according to Gibson et al. (1994) at the R6 growth stage. Disease incidence was based on the percentage of plants with foliar symptoms. Foliar disease severity was recorded as: 1= 0 to 10% chlorosis or 1 to 5% necrosis, 2 = 10 to 20% chlorosis or 6 to 10% necrosis, 3 = 20 to 40% chlorosis or 10 to 20% necrosis, 4 = 40 to 60% chlorosis or 20 to 40% necrosis, 5 = > than 60% chlorosis or > than 40% necrosis, 6 = up to 33% defoliation, 7 = up to 66% defoliation, 8 = > than 66% defoliation and 9 = premature death of the plant. A disease index (DX) was calculated as (DI x DS)/9.

Greenhouse experiments

The two sets of RILs previously described, along with the parents and controls were evaluated for foliar resistance to SDS in the greenhouse using the cone inoculation method. One hundred ml of steam-treated soil mix (2:1 sand:soil) was placed in a SC-10 type cone and 5 ml (3 g) of FSG infested white sorghum seeds were placed on the top of the soil mix. Twenty ml of soil mix were added to cover the infested seeds, and three soybean seeds were added to each cone, which were covered with another 20 ml of soil mix.

After emergence, seedlings were thinned leaving one seedling per cone. The soil was maintained to near the water-holding capacity by flooding the cones twice a day. The cone was the experimental unit and these were arranged in an RCBD with eight replicates. For the PI 567374 x Omaha population, two experiments with four replicates each were planted one month apart. For the Ina x LN91-1695 population, the eight replicates were planted at the same time. The experiments were done during the winter of 2003. The plants were rated three weeks after germination using a scale from 1 (no symptoms) to 6 (severe symptoms) based on leaf chlorosis and necrosis, defoliation, and premature plant death. The rating scale was the following: 1 = no symptoms, 2 = slight symptom development, with chlorosis on leaves (1 to 20% foliage affected), 3 = moderate symptom development, with chlorosis and necrosis on leaves (20 to 40% foliage affected), 4 = heavy symptom development, with chlorosis and necrosis on leaves (40 to 60% foliage affected), 5 = severe symptom development, with chlorosis and necrosis on leaves (60 to 80% foliage affected), 6 = severe symptom development, with chlorosis and necrosis on leaves (more than 80% foliage affected).

FSG inoculum production

The Monticello isolate of FSG, (originated from Monticello, IL) was the source of all the inoculum used in the field and greenhouse. The grain inoculum was prepared by first soaking seed of white sorghum overnight. Four kg of sorghum seed were placed into a clear autoclave bag and autoclaved for 1 hour twice. Each bag was then inoculated with 30 plugs (4 mm diameter) of fungal mycelium and incubated at room temperature for two weeks. The colony forming units (CFU) of the infested seeds was determined as previous reported on hairy roots (Li et al., 2002) with slight modification. Briefly, 1 g of sorghum inoculum was soaked in a 250-ml Erlenmeyer flask containing 100 ml of sterile distilled water. The flasks were shaken at 150 rpm on an orbital Shaker for 30 min, and then serially diluted 10 fold with sterile distilled water twice. From each dilution, 100 µl of inoculum dilution was spread on an agar plate (100 x 15 mm) containing *Fusarium solani* f. sp. *glycines* semi-selective medium (Huang and Hartman, 1996). Six plates were used for each inoculum dilution. The plates were incubated at room temperature (25 ± 2ºC) for 10 days. Colonies of *Fusarium solani* f. sp. *glycines* were identified as described previously (Li et al., 2000). The number of colonies on each plate was used to determine the colony-forming units per g of sorghum. The experiment was conducted two times.

DNA marker analysis

DNA was isolated from leaf samples of ten plants from each line in the population using the CTAB extraction method (Keim and Shoemaker, 1988) with slight modifications. Simple sequence repeat (SSR) markers developed by P. B. Cregan (USDA-ARS, Beltsville, MD) were used according to methods described in Cregan and Quigley (1997) to genotype the lines. Polymerase chain reaction products were analyzed in non denaturing polyacrylamide gels (Wang et al., 2003). Markers identified by Hnetkovsky et al. (1996), Chang et al. (1996), Iqbal et al.

(2001) and Nijti et al. (2001) as associated with SDS resistance in Forrest, Essex, Pyramid and Douglas were initially used to genotype the lines. Both populations were then evaluated with additional markers.

Statistical analysis

An analysis of variance for main effects of RILs, years and their interaction for the recorded field data and for main effects of RILs, experiments and their interaction for the recorded greenhouse data were computed using the PROC mixed of SAS (SAS Institute, 2000). Years, experiments, lines and blocks were treated as random factors. Correlations were calculated between field and greenhouse data using the CORR procedure of SAS. The broad sense heritability for each year and across years for field experiments and for each experiment and across experiments for the greenhouse data was determined according to Hallauer and Miranda Filho (1988).

A linkage map was made with the marker data using Joinmap 3.0 (Van Ooijen and Voorrips, 2001). Quantitative trait loci controlling SDS resistance were mapped with the software MapQTL 4.0 (Van Ooijen et al., 2002) using composite interval mapping (CIM) (Jansen and Stam, 1994; Zeng, 1994). To determine experimentwise LOD thresholds for the QTL analysis, 1000 permutations were run in MapQTL. The total R^2 of the phenotypic variation explained by all markers associated with resistance in a population was calculated with ANOVA using the GLM procedure of SAS (SAS Institute, 2000).

RESULTS AND DISCUSSION

PI 567374 x Omaha population

Analysis of greenhouse data

Data from the field evaluation of the PI 567374 x Omaha population were not used in the QTL analysis because of the great range of maturity of the RILs (54 days) and the high degree of susceptibility of the RILs to foliar diseases. This made it impossible to accurately rate the SDS resistance of the RILs in the field. Therefore, only the SDS seedling resistance ratings were used to map QTL.

Typical SDS symptoms were observed on plants in the greenhouse experiment. The analysis of variance of the greenhouse experiments 1 and 2 (Table 3.1) and the pooled analysis (Table 3.2) showed a significant effect for RILs, indicating that there was significant ($P< 0.001$) genetic variability among the RILs for DS. The mean DS for the resistant parent PI 567374 was 1.7 (Table 3.3), similar to the resistant control Ina (DS=2.4). The susceptible parent Omaha showed a DS of 5.3, similar to the susceptible control Spencer, which showed a DS of 5.4. Heritability was estimated as 36% for experiment 1 and 62% for experiment 2. The mean squares for RILs in experiment 1 was lower than the mean squares for RILs in experiment 2, which led to the relatively low heritability for experiment 1. Across experiments, the heritability was estimated as 45%. These rates are similar to those reported by Njiti et al. (2001), who found heritabilities ranging from 33% to 66% for greenhouse evaluations of a population derived from the cross Forrest x Essex. Fronza et al. (2003) found heritabilities ranging from 33% to 62% for a population derived from a cross between the cultivars Conquista and Estrela, which was evaluated in the greenhouse.

The histogram of the RILs for DS (Figure 3.1) showed that the distribution for DS was continuous and normal. Continuous distribution is an indication of polygenic inheritance for SDS resistance in this population. None of the RILs showed greater resistance than the parent PI567374, indicating that the susceptible parent Omaha has no or few beneficial SDS resistance alleles. The cultivar Omaha showed the highest DS of the experiment.

DNA marker analysis

The entire population was genotyped with 112 SSR markers covering all 20 linkage groups (LG) of the soybean genetic linkage map (Song et al., 2004). Sixty one markers grouped into 17 LGs and 51 markers remained unlinked.

Markers associated with DS in the greenhouse

None of the genetic regions where SDS resistance QTL were previously reported in the literature (Hnetkovsky et al., 1996; Chang et al., 1996; Nijti et al., 1996; Njiti et al., 2000; Igbal et al., 2001) were significantly associated with DS in the population. This suggests that PI 567374 has different SDS resistance genes than those identified in other studies. Another hypothesis is that the previously mapped QTL were common to both parents of my population and therefore, were not segregating. However, this was unlikely because Omaha was very susceptible to SDS, with greenhouse scores similar to the susceptible check.

Six chromosomal regions were associated with DS at $\alpha = 0.05$ in this population (Table 3.4). The 5% significance level might be considered a high threshold, increasing the probability of declaring false positive QTL (Type I error). The appropriate significance level to use depends on the purpose of QTL mapping. If the goal is to exploit QTL information to do marker-assisted

selection for a complex trait, type II errors increase in importance. In this situation, a less stringent significance level is appropriate (Dudley, 1993).

Two chromosomal regions were found to contain QTL significantly associated with DS at P< 0.005. The first region was located on LG D2, identified by the marker Satt311 (P = 0.0032, R^2 = 12%). The beneficial allele was derived from PI 567374. This region has been previously reported as associated with SDS DI in a population derived from a cross between the cultivars Ripley and Spencer (Brian Diers, personal communication). This region also has been reported to be associated with resistance to white mold (Arahana et al., 2001) and resistance to race 14 of soybean cyst nematode (SCN) (Shuster et al., 2001). Associations between SCN resistance and SDS resistance have been observed (Melgar et al., 1994; Scherm et al., 1998) and there is evidence that root wounding by SCN infection may promote root infection from FSG. However, it is unlikely that this was a factor in my study, which was carried out in the greenhouse in sterilized soil that was not inoculated with SCN.

The second region was located on LG I and was identified by the marker Sat_299 (P = 0.0009, R^2 = 12%). The beneficial allele was derived from PI 567374 for this marker. A QTL for SDS resistance was previously mapped to LG I in the Forrest x Essex population (Igbal et al., 2001). However, this QTL was mapped over 50 cM from the SDS resistance QTL in my population. A region close to my resistance QTL was reported to be associated with canopy width (Mian et al., 1998). It is unlikely that the same QTL controls both SDS resistance and canopy width through pleiotropy. The markers Satt311 and Sat_299 together explained about 21.3 % of the variation for DS.

Four SDS resistance QTL were mapped using a significant threshold between P < 0.05 and P < 0.005. The first was identified with marker Satt547 (P = 0.01, R^2 = 10%) on LG J where

QTL loci associated with soybean cyst nematode (Concibido et al., 1997) and brown stem rot (Klos et al., 2000) resistance were previously mapped. Quantitative trait loci for white mold [*Sclerotinia sclerotiorum* (Lib) de Bary] resistance on LG L and LG O were previously mapped (Aruana et al., 2001) close to the regions where we identified SDS resistance with Satt462 on LG L ($P = 0.04$, $R^2 = 4\%$) and Satt153 on LG O ($P = 0.04$, $R^2 = 7\%$). The final SDS resistance QTL was mapped to LG C1 by Satt399 ($P = 0.01$, $R^2 = 9\%$). The beneficial alleles for all QTL were from PI 567374.

No QTL were found to be significantly associated with DS based on CIM (Table 3.5), using a threshold of 2.3, which give us an experiment wise threshold of 0.05 based on a permutation test. Higher LOD scores were found for experiment 2 than for experiment 1, which is likely due to the higher heritability observed in experiment 2 than in experiment 1.

The identification of these QTL for DS seedling resistance will be useful for soybean breeders developing new partially resistant cultivars through marker-assisted breeding. PI 567374 has shown a high level of both field and greenhouse SDS resistance and it is an important source of resistance to the disease. Additional research is still needed to identify more SDS resistance QTL by testing the population with more markers and to confirm the QTL we mapped.

Ina x LN91-1695 population

Analysis of field and greenhouse data

Field experiments were planted during 2002, 2003 and 2004, and typical SDS symptoms were observed on plants in 2003 and 2004 (Table 3.6). In 2002, only slight SDS symptoms were observed, possibly because the soybean seeds were planted two weeks after the field was inoculated with the FSG infested sorghum. In addition, 2002 was the first year the field was

inoculated with FSG and the field had no history of major SDS outbreaks. Hence, the data were not used for the QTL analysis. The pooled analysis of variance of the field 2003 and 2004 experiments (Table 3.2) showed a significant effect of RILs (P< 0.001) for DI, DS and DX. The RIL x environment interaction also was significant for all SDS resistance traits. This was expected because of the sensitivity of SDS symptom development to environmental factors (Gibson et al., 1994; Nijti et al., 1996). Heritabilities across 2002 and 2003 were estimated as 59% for DI, 42% for DS and 62% for DX (Table 3.6), which are less than those reported in the literature. Chang et al (1996) observed heritabilities of 89% for DI and 76% for DS for the Forrest x Essex population evaluated across five environments. Njiti et al. (2001) reported a heritability of 75% for DI for a population derived from a cross between Pyramid and Douglas evaluated across five environments.

The DX of RILs in the population was highly skewed toward low DX values (Figure 3.2). We don't have a clear explanation for the skewing of the distribution. One hypothesis is that there might be two or more QTL for SDS resistance derived from the resistant cultivar Ina and any one provides a good level of resistance to the disease. In this case, only the individuals that are homozygous susceptible for all QTL would have a high level of foliar SDS symptoms. The cultivar Ina showed no SDS symptoms across the two environments while the susceptible parent LN91-1695 showed a mean DX of 11.9.

Typical SDS symptoms were observed on plants in the greenhouse experiment. Similar to the results from PI 567374 x Omaha population, the frequency distribution for DS was continuous and normal (Figure 3.3). The analysis of variance (Table 3.1) showed that there were significant differences among RILs (P< 0.001) for DS. The overall mean for DS was 2.9 (Table 3.6), which is lower than the overall mean DS for the PI 567274 x Omaha population. This lower

DS is probably because of the greater susceptibility observed for Omaha compared to LN91-1695. Heritability for the greenhouse test was estimated as 45%, which is lower than the heritability observed for the field experiment. This was not expected, since in the greenhouse there was a better control of the environment than in the field. Despite the better control of the environment in the greenhouse, the field experiments could separate the lines better than the greenhouse experiments resulting in higher heritability for the field experiments.

DNA marker analysis

The entire population was genotyped with 86 SSR markers covering all 20 linkage groups (LG) of the soybean genetic linkage map (Song et al., 2004). However, only 71 markers showed polymorphism across the whole population. Forty seven of those markers were grouped into seven LG and 24 markers remained unlinked. The remaining 15 were fixed in the population for the allele from Ina, although the parents were polymorphic. A similar lack of segregation has been observed in other soybean mapping studies (Friedrich Kopisch, Peter Guzman, personal information), but not in the high frequency of my study. I don't have a good explanation for this lack of polymorphism. Rahman (2001) observed a distorted segregation for petal color in a backcross population of rape seed (*Brassica rapa*). The author suggested that selection for male gametes might have occurred, which resulted in a skewed segregation in the backcross population. However, if segregation distortion was occurring in my population because of selection, we would expect the distortion to be limited to specific genomic locations where the selection occurred. In my case, the non segregating markers were spread throughout the genome.

Markers associated with DX in the field and DS in greenhouse

Five chromosomal regions were associated with field DX at $\alpha = 0.05$ in this population (Table 3.7). The markers Satt270 (P = 0.0028, R^2 = 12.7%) and Satt354 (P= 0.0072, R^2 = 7.8%) both mapping within 4 cM on LG I showed the most significant association with DX. The resistance allele for this QTL was from Ina. A SDS resistance QTL had been previously mapped from the cultivar Essex (Iqbal et al., 2001) with Satt354. A second region on LG I associated with DX was mapped in my population by Sct_189 (P= 0.0139, R^2 = 9.6%) and the beneficial allele was derived from LN91-1695. This region has not been previously reported as associated with SDS resistance. The marker Sct_189 is located 67 cM below Satt354 and 14 cM below Sat_299, where a SDS resistance QTL was mapped in the PI 567374 x Omaha population.

Another region associated with DX was mapped onto LG C2 by Satt371 (P= 0.02 R^2 = 12%) and the beneficial allele for this QTL was from Ina. A SDS resistance QTL was previously mapped to this region in the Forrest x Essex population (Igbal et al., 2001) and the beneficial allele was from the cultivar Essex. Ina and Essex are not closely related, but since the genetic base of the American soybean germplasm is narrow (Carver et al., 2004) these cultivars may have alleles in common. On the other hand, Ina and Forrest are closely related and most SDS resistance QTL regions were first mapped from cultivar Forrest and located on LG G (Chang at al., 1996, Iqbal et al., 2001, Njiti et al, 2001). However, none of the LG G alleles were mapped on my population. The resistance allele for these previously mapped QTL might be common for both parents of my study, or Ina doesn't have the SDS resistance alleles from Forrest.

Two additional regions associated with DX on my population were mapped onto LG A1 by Satt684 (P= 0.02 R^2 = 8.5%) and on LG E by Satt268 (P = 0.01, R^2 = 9.8%). Both regions derived their beneficial alleles from Ina. Soybean cyst nematode QTL were previously reported

on LG A1 (Vierling at al., 1996) and on LG E (Wang et al., 2001) close to the regions we mapped the QTL for SDS resistance.

My study showed that three out of five QTL mapped with the field data were also mapped with the greenhouse data, which is due to the good correlation ($r=0.51$, $P<0.001$) between field DX and greenhouse DS. This is consistent with the results from the second chapter of this thesis. No significant markers were identified with the CIM analysis with a LOD score threshold = 2.0 based on the permutation test, likely because of the limited number of markers tested in the population.

The identification of these QTL on my population will be useful for a marker-assisted selection program for developing new SDS resistance cultivars. The identification of the QTL on LG I by Satt354 and Sat_299 and the QTL on LG C2 by Satt371 was important as this is the first confirmation of these QTL. The cultivar Ina also has shown a great level of both field and greenhouse SDS resistance along with other important agronomic traits, and it is an important source of resistance to SDS.

Table 3.1. Sources of variation, degrees of freedom (df), mean squares, and significance values for disease severity (DS) in two soybean populations inoculated with *Fusarium solani* f. sp. g*lycines* in the greenhouse.

Source of variation	PI 567374 x Omaha				Ina x LN91-1695	
	Exp 1		Exp 2			
	df	Mean square DS	df	Mean square DS	df	Mean square DS
RILs	95	2.5***	95	4.2***	93	2.4***
Rep	3	2.0 ns	3	1.2 ns	7	3.4**
Residual	268	1.6	270	1.6	589	1.4

*, **, ***,ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 3.2. Sources of variation, degrees of freedom (df), mean squares, and significance values for SDS disease severity (DS) in the PI 567374 x Omaha population inoculated with *Fusarium solani* f. sp. g*lycines* in the greenhouse and for SDS disease incidence (DI), disease severity (DS) and disease index (DX) for the Ina x LN91-1695 population inoculated with *Fusarium solani* f. sp. g*lycines* in the field.

Source of variation	PI 567374 x Omaha		Ina x LN91-1695			
		Mean square		Mean square		
	df	DS	df	DX	DI	DS
Exp	1	5.4 ns	1	0.008 ns	0.064 ns	60.7***
Rep	7	0.9	2	210.6**	842.7***	6.9*
RILs	95	4.5***	93	127.8***	581.6***	5.6***
RILs * Exp	95	2.2*	93	36.5***	174.1***	2.1*
Residual	537	1.6	374	18.6	104.2	1.6

*, **, ***,ns: Significant at the 0.05, 0.01, 0.001 probability levels and not significant, respectively.

Table 3.3. Means, range and broad sense heritability (h^2) estimates for disease severity (DS) for 96 F_4-derived lines, parents and controls inoculated with *Fusarium solani* f. sp. g*lycines* in the greenhouse.

		Exp 1		Exp 2		Pooled
Parents						
PI 3567374		1.7		1.6		1.7
Omaha		5.2		5.4		5.3
Controls						
Ina (resistant)		3.3		1.8		2.4
Spencer (susceptible)		5.7		5.2		5.4
Population						
Mean		3.7		3.8		3.7
Range		1.6 – 5.7		1.6 -6.0		1.6 - 5.6
Heritability (%)		36		62		45

Table 3.4. Linkage group (LG), significance values, percentage of the phenotypic variation for sudden death syndrome (SDS) resistance (R^2 %) explained by the markers and means of SDS disease severity (DS) for each marker associated with seedling SDS resistance in the PI 567374 x Omaha population.

				Mean DS[+]	
Marker	LG	P>F	R^2 (%)	PI 567374	Omaha
Satt399	C1	0.0141	9	3.53	4.01
Satt311	D2	0.0032	12	3.37	3.94
Sat_ 299	I	0.0009	12	3.49	4.05
Satt547	J	0.014	10	3.51	4.02
Satt462	L	0.041	4	3.68	3.94
Satt153	O	0.039	7	3.53	3.99

[+]Mean DS of the homozygous classes for each marker.

Table 3.5. Linkage group (LG), significance values, percentage of the phenotypic variation for sudden death syndrome (SDS) resistance (R^2 %) explained by the markers, LOD scores, and percentage of resistance explained by the QTL based on composite interval mapping for each marker associated with seedling SDS resistance in the PI 567374 x Omaha population.

Experiment	P>F	R^2 (%)	LOD*	QTL % expl.
----Satt311 LG D2 ----				
Pooled	0.0032	12	1.5	6.2
Exp 1	0.1044	4	0.4	2
Exp 2	0.0026	12	1.8	7.3
----Sat_ 299 LG I----				
Pooled	0.0009	12	2.0	8.7
Exp 1	0.0277	6	0.6	3
Exp 2	0.0011	11	2.2	9.3

*LOD score threshold based on permutation test =2.3

Table 3.6. Means, ranges, and broad sense heritability (h^2) estimates for sudden death syndrome (SDS) disease incidence (DI), disease severity (DS) and disease index (DX) for 92 F_4-derived recombinant inbred lines (RILs) from the cross Ina x LN91-1695, parents and controls inoculated with *Fusarium solani* f. sp. g*lycines* (FSG) in the field, and for SDS disease severity (DS) evaluated in the greenhouse.

	Field Experiment			
Trait	Exp 2003	Exp 2004	Pooled	Greenhouse
		Ina		
DI	0.0	0.0	0.0	
DS	0.0	0.0	0.0	2.1
DX	0.0	0.0	0.0	
		Ripley (control)		
DI	0.6	0.3	0.5	
DS	0.7	0.7	0.7	1.6
DX	0.04	0.02	0.03	
		LN 91-1695		
DI	31.6	23.3	27.5	
DS	4.1	3.6	3.88	4.7
DX	14.6	9.3	11.9	
		Spencer (control)		
DI	73.3	37.5	55.4	
DS	5.4	5.2	5.3	5.8
DX	44.6	21.5	33.0	
		Overall mean of the population		
DI	9.6	9.6	9.63	
DS	2.1	2.7	2.33	2.9
DX	3.6	3.6	3.6	
		Range of the population		
DI	0 - 46.7	0 - 48.3	0 - 36.7	
DS	0 - 4.6	0 - 4.5	0 - 4.6	1.6 - 5.8
DX	0 - 26.4	0 - 19.3	0 - 18.9	
		Heritability (%) of the population		
DI	82	54	59	
DS	47	43	42	45
DX	59	85	62	

Table 3.7. Linkage group (LG), significance values, percentage of the phenotypic variation for sudden death syndrome (SDS) resistance (R^2 %) explained by the markers and means of SDS disease severity (DS) for each marker associated with SDS resistance in the Ina x LN91-1695 population inoculated with *Fusarium solani* f. sp. g*lycines* in the greenhouse and in the field.

	Field experiments					Greenhouse experiment			
				Mean DS[+]				Mean DS[+]	
Marker	LG	P>F	R^2 (%)	Ina	LN91-1695	P>F	R^2 (%)	Ina	LN91-1695
Satt684	A1	0.02	8.5	1.9	4.3	0.026	8.5	2.7	3
Satt371	C2	0.02	12	2.6	3.6	0.99	0.2	2.9	2.9
Satt268	E	0.0102	9.8	2.5	4.6	0.0021	12	2.7	3.1
Sct_189	I	0.0139	9.6	5.1	2.6	0.36	1	2.9	2.8
Satt354	I	0.0072	7.8	2.3	4.5	0.113	2	2.8	2.9
Sat_270	I	0.0028	12.7	2.4	5.4	0.04	6.8	2.7	3.1

[+]Mean DS of the homozygous classes for each marker.

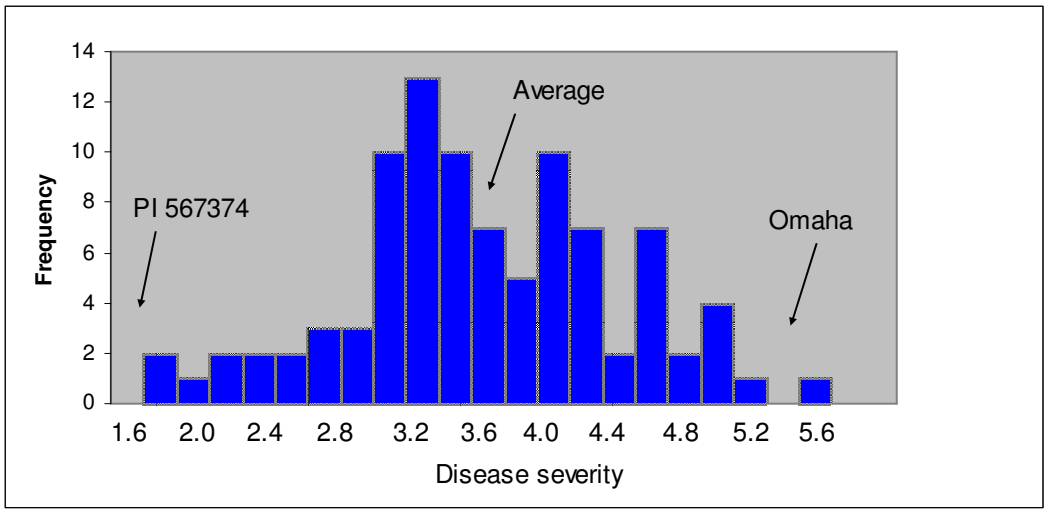

Figure 3.1. Histogram for 96 F_4- derived RILs segregating for sudden death syndrome disease severity (DS) in the greenhouse. The population was developed from crossing PI 567374 and Omaha. The DS means for PI 567374, Omaha and the population are denoted by the arrows.

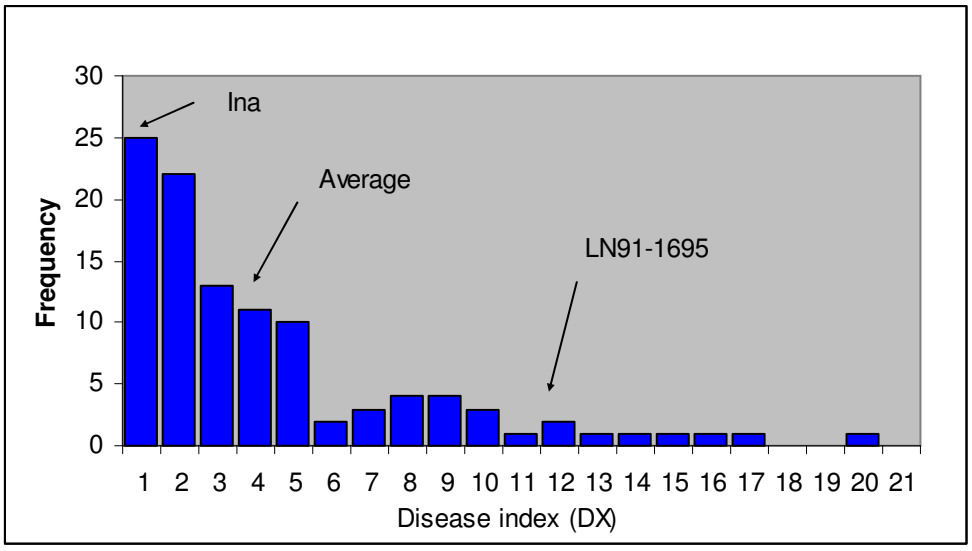

Figure 3.2. Histogram for 92 F_4- derived RILs segregating for sudden death syndrome disease index (DX) in the field. The population was developed from crossing Ina and LN91-1695. The DX means for Ina, LN91-1695 and the population are denoted by the arrows.

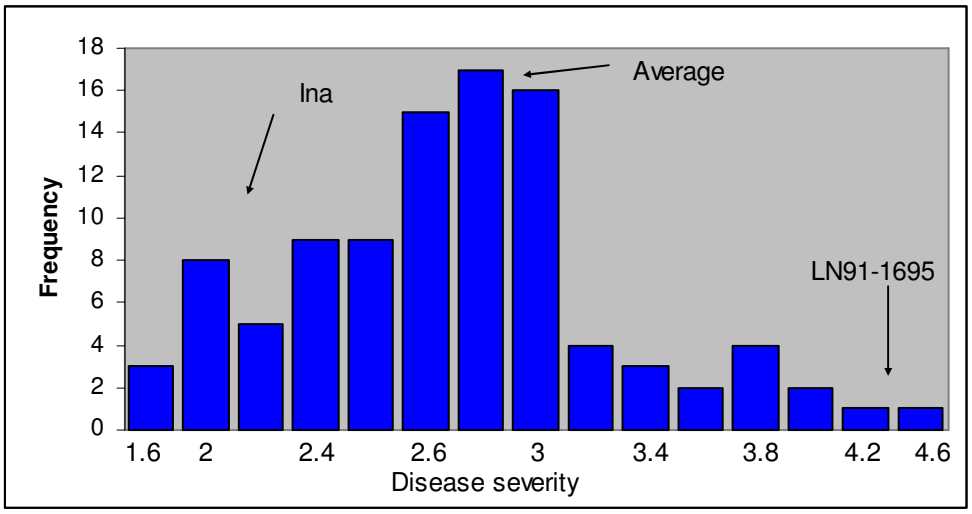

Figure 3.3**.** Histogram for 92 F_4-derived RILs segregating for sudden death syndrome disease severity (DS) in the greenhouse. The population was developed from crossing Ina and LN91-1695. The DX means for Ina, LN91-1695 and the population are denoted by the arrows.

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