Fusarium root rot of soybean – occurrence, impact, and relationship with soybean cyst nematode

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Introduction

Fusarium species are ubiquitous soilborne pathogens that can cause devastating and difficult-to-manage damping-off, root rot, wilt, or sudden death syndrome (SDS) in soybeans (Armstrong, 1950; French and Kennedy, 1963; Rupe, 1989). At least 12 different species of *Fusarium* have been reported to be associated with soybean roots (McGee, 1992); among these species and the diseases they cause, the economic impact is widely recognized only for SDS; economic impacts of *Fusarium* wilt and root rot are much less well documented. *Fusarium* root rot is widespread in US (Yang and Feng, 2001). *F. oxysporum* complex and *F. solani* complex are generally believed to be the major species causing root rot symptoms (Nelson, 1999), but other species may be pathogenic. These *Fusarium* species are not well identified and no comprehensive evaluations of their impact on yield have been undertaken, and their pathogenic capabilities are extremely variable. These knowledge gaps seriously hinder efforts to manage the diseases effectively. Observations suggest that there are interactions among *Fusarium* root rot and soybean cyst nematode (*Heterodera glycines*) (Nelson, 1999), but these interactions have not been verified or measured. The variable nature of *Fusarium* root rot occurrence may be better explained through more detailed knowledge of the important species and clarification of the importance of SCN-*Fusarium* interactions. Understanding this variability is an important step toward effective management of the disease.

To have a better understanding of the importance of root-infecting *Fusarium* species in soybean productivity in Iowa, a root survey, greenhouse and field experiments were conducted in order to accomplish the following objectives, 1) characterize the frequency and aggressiveness of *Fusarium* species associated with symptomatic soybean roots in Iowa; 2) estimate their impact on soybean growth and yield; and 3) determine whether SCN infestation enhances Fusarium root rot in soybean. Systematic sampling of soybean roots from fields was followed by characterization of Fusarium species using morphological features and molecular approaches. Greenhouse experiments were used to assess species virulence, and field experiments were conducted to estimate yield loss caused by *Fusarium* root rot and assess the impact of SCN infestation on *Fusarium* root colonization and rot. This information is needed to guide and improve management practices for the *Fusarium* complex and soybean cyst nematode. The results of this study will provide crucial information on the biology, prevalence and distribution of the disease in Iowa, which can help guide management efforts and future research, including studies of the genetic diversity within species, epidemiological and ecological features of the disease, and host-pathogen interactions.

Material and methods

Frequency of Fusarium species

In collaboration with twelve Iowa State University Extension field specialists, soybean roots were collected during three years for isolation of *Fusarium* species from 3 fields in 98 Iowa counties. Ten plants were collected from each field at both V2-V3 and R3-R4 growth stages. Soybean root pieces were superficially sterilized and plated onto a *Fusarium* spp. selective media. *Fusarium* colonies were transferred to carnation leaf agar (CLA) and potato dextrose agar (PDA) and identified to species based on cultural and morphological characteristics. Species identities were confirmed for selected isolates by amplification and sequencing of the elongation factor gene alpha (EF1-alpha) region. Frequency and distribution of *Fusarium* species among locations and sampled times were documented.

Pathogenicity test

Representative isolates from the predominant *Fusarium* species found during the root survey were tested in greenhouse conditions on soybean. Soybean seeds of AG2306 variety were planted in sterile soil infested with each *Fusarium* isolate and maintained at greenhouse conditions in water baths at ± 18°C. Root rot severity, shoot dry weight and root dry weight were measured at V3 stage. The effect of Fusarium isolates on root growth was assessed using a root scanner (Epson Perfection V700 photo) that determines length, diameter and root discoloration or rotted area.

Microplots field experiments

Two-year microplot experiments were established at the ISU Horticulture Research Farm and the ISU Hinds Farm near Ames, Iowa, to estimate potential yield loss caused by some *Fusarium* species isolates used at the greenhouse pathogenicity test. Microplots consisted of rectangular plots containing a single row of soybean plants. Inoculum was prepared by inoculating sorghum seed with most frequent *Fusarium* isolates from soybean roots. Plots were infested with isolates from seven *Fusarium* species (*F. oxysporum, F. solani, F. graminearum, F. acuminatum, F. semitectum, F. equiseti and F. sporotrichioides*). At growth stage R1, a subsample of soybean plants was removed from each microplot to evaluate root rot symptoms, root dry weight and shoot dry weight. The effect of *Fusarium* isolates on root growth was evaluated using the root scanner. At harvest, yield and seed moisture content and size were measured.

SCN -Fusarium interaction

In order to test the interaction between SCN and *Fusarium* in greenhouse conditions, 16 *Fusarium* isolates from 8 *Fusarium* species were used. A factorial experiment combined two soybean lines (susceptible Pioneer 92M91 and SCN-resistant Pioneer 92M54) grown in soil inoculated with *Fusarium* alone, SCN alone, and a combination of both pathogens. Number of SCN females, root rot severity, root dry weight, shoot dry weight, and root length, discoloration and biomass were measured after 6 weeks. Experiment was maintained in water bath conditions at 24°C.

Results and discussion

Frequency of Fusarium species and microplots experiments

A total of 11 *Fusarium* species have being isolated from soybean roots in Iowa. In general, the most frequent and widespread species were the *F. oxysporum complex, F. solani complex, F. acuminatum*, and *F. graminearum*, each recovered from 5%-30% of root samples (Fig. 1). Other species such as *F. semitectum, F. equiseti, F. sporotrichioides, F. proliferatum, F. virguliforme, F. subglutinans, F. verticillioides* and unknown *Fusarium* spp. were less frequent. Species prevalence among fields differed regionally (Table 1). Some species (*F. virguliforme, F. subglutinans* and *F. verticilloides*) were recovered from a low percentage of fields and found in less than 5 counties. Most of the species have been reported previously, but several (*F. subglutinans, F. sporotrichioides, F. semitectum*) have not previously been associated with soybean roots. Differences in species frequency by growth stage were found, higher amount of species were recovered from roots at V2-V3 stage, and lower amount from root at R3-R4 stages (Fig.2)

Pathogenicity test and microplots field experiments

Greenhouse assay experiments were conducted using 49 Fusarium isolates, in order to determine the aggressiveness of *Fusarium* species isolated from Iowa soybeans. Soybean seeds were planted in sterile sand–soil (2:1) inoculated with fungus and maintained in water baths at 18±1C in the greenhouse. Root rot severity, shoot dry weight and root dry weight were measured at stage V3. In general, pathogenicity tests showed differences in aggressiveness among *Fusarium* isolates (Fig. 3). Root rot severity differed among isolates with *F. graminearum* isolates causing the most severe root rot symptoms; followed by *F. proliferatum, F. sporotrichioides* and *F. virguloforme* isolates. Significant variation in aggressiveness was observed among *F. oxysporum* isolates and *F solani* isolates.

On the other hand, microplot experiments results show that inoculation with *Fusarium* isolates affected shoot dry weight and root dry weight in all locations. However, there were no significant differences among *Fusarium* isolates in terms of yield, seed size and seed moisture in general. Nevertheless, regression analysis by isolate showed that there is an interaction between root rot severity and yield and, between root rot severity and seed size, and root severity and root dry weight in some of the *Fusarium* isolates tested at two different locations (Fig 4.)

Figures 4a, 4c and 4e illustrate the relationship between disease severity and yield (ka/ha) for different *Fusarium* isolates. Results indicated *F. acuminatum* (FA3) has the best relation to yield. Variable severity for this sampling unit explained 92.98% of the variation in yield (kg/ha). Isolate with less relationship with yield was *F. equiseti* (FE1), root rot severity for this sampling unite explained 44.79% of the variation in yield. On the other hand, only isolates FA1 and FE1 showed a relationship between root rot severity and seed size and only root rot severity caused by FA3 explained variation in root dry weight.

SCN -Fusarium interaction

Greenhouse studies suggest that there may be a significant interaction between SCN and some *Fusarium* isolates causing root rot. Root rot severity, shoot dry weight and root dry weight differed among *Fusarium* isolates (table 2). In general means for root rot severity were greater on plants co-inoculated with *Fusarium* and SCN, than on plants inoculated with *Fusarium* alone. There was a significant interaction between SCN and *Fusarium* isolates in root rot and root dry weight (table 2). Co-inoculation with SCN resulted in greater root rot severity on plants inoculated with only seven *Fusarium* isolates (FG2, FO1, FO2 FS2, FSE1 FSP1 and FSP2) for both soybean varieties (Fig. 5). Results also showed there is an interaction between SCN and *Fusarium oxysporum* isolates, due to presence of nematode increase disease severity in roots. In general, it was observed a greater effect of nematode co-inoculation with *Fusarium* in root rot (P=0.0004), and shoot dry weight (0.0166) in resistant than in susceptible soybeans.

References

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Table 1. Prevalence and incidence of *Fusarium* species in 55 fields located in 50 counties in Iowa, 2007

Species	Prevalence (%)	Incidence (%)
F. oxysporum	56.4	28.4
F. solani	56.4	23.7
F. acuminatum	50.9	20.5
F. graminearum	34.5	16.1
F. semitectum	20	3.8
F. equiseti	9.1	2
F. proliferatum	9.1	2
F. soporotrichoides	5.4	0.9
F. subglutinans	7.3	1.2
F.virguliforme	5.4	1.5

Table 2. P values for effects of *Fusarium* isolates, varieties, SCN inoculation and their interactions on root rot severity, root dry weight, shoot dry weight in soybeans.

	Root rot severity (%)	Root dry weight (g)	Shoot dry weight (g)
Repetition	0.2604	0.3412	0.8394
Isolate (Iso)	<.0001*	<.0001*	<.0001*
SCN	0.4418	<.0001*	0.0003*
Iso* SCN	<.0001*	0.0328*	0.0053*
Variety (Var)	0.1078	<.0001*	0.0013*
Iso * Var	0.0388*	0.0097*	0.3173*
SCN*Var	0.2726	0.0097*	<.0001*

Values are based on one experiment with 5 replicates of each isolate per each cultivar

*: significant differences at 0.05 level

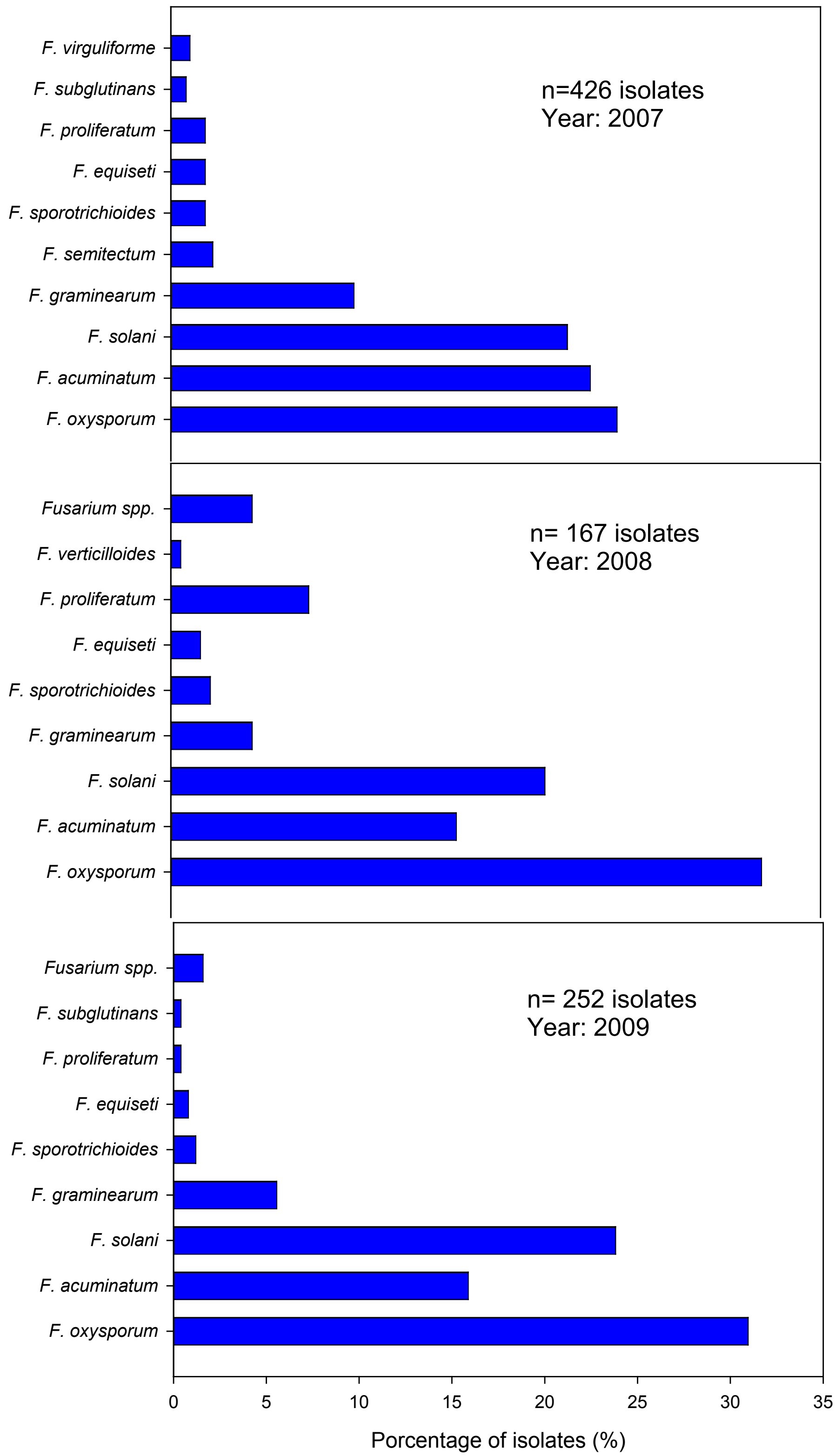

Figure 1. Frequency of *Fusarium* species associated with soybean roots in Iowa in 3 year root survey in Iowa .

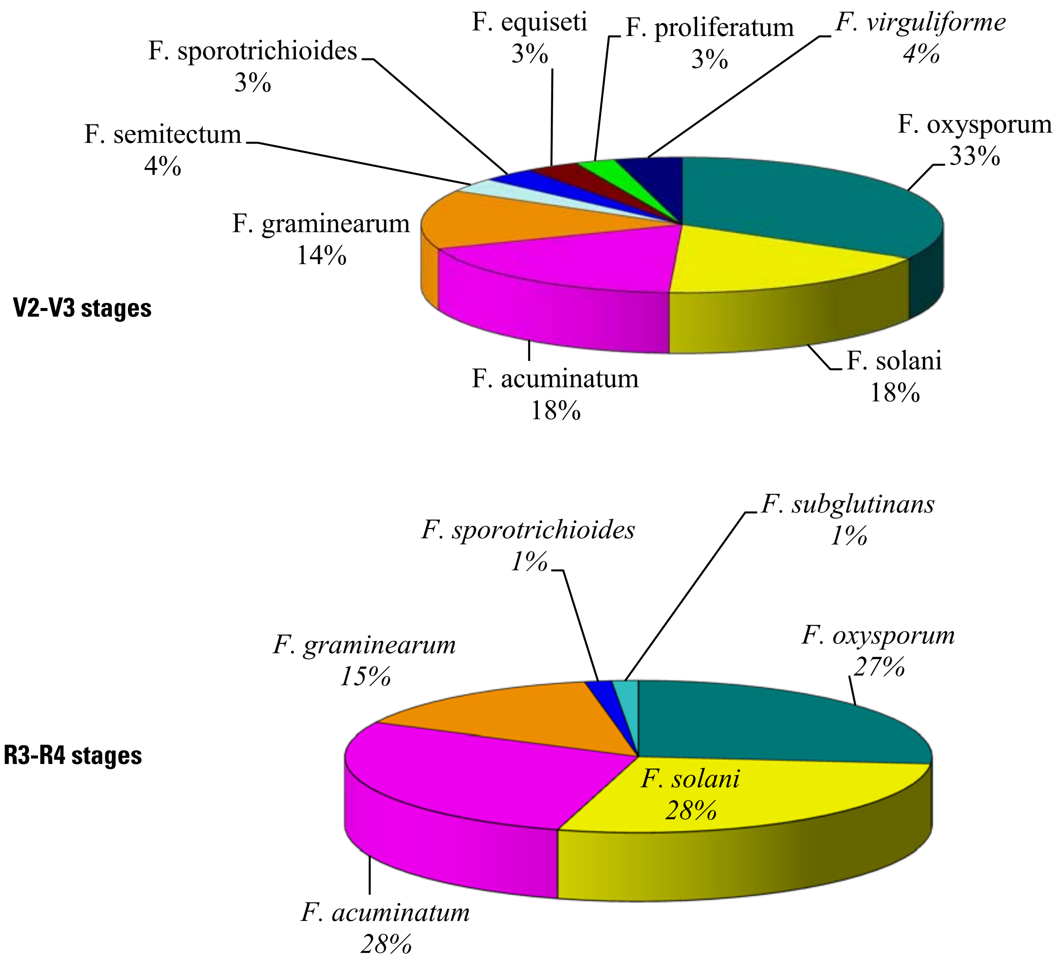

Figure 2. Frequency of *Fusarium* species at two soybean stages in 2007 in Iowa.

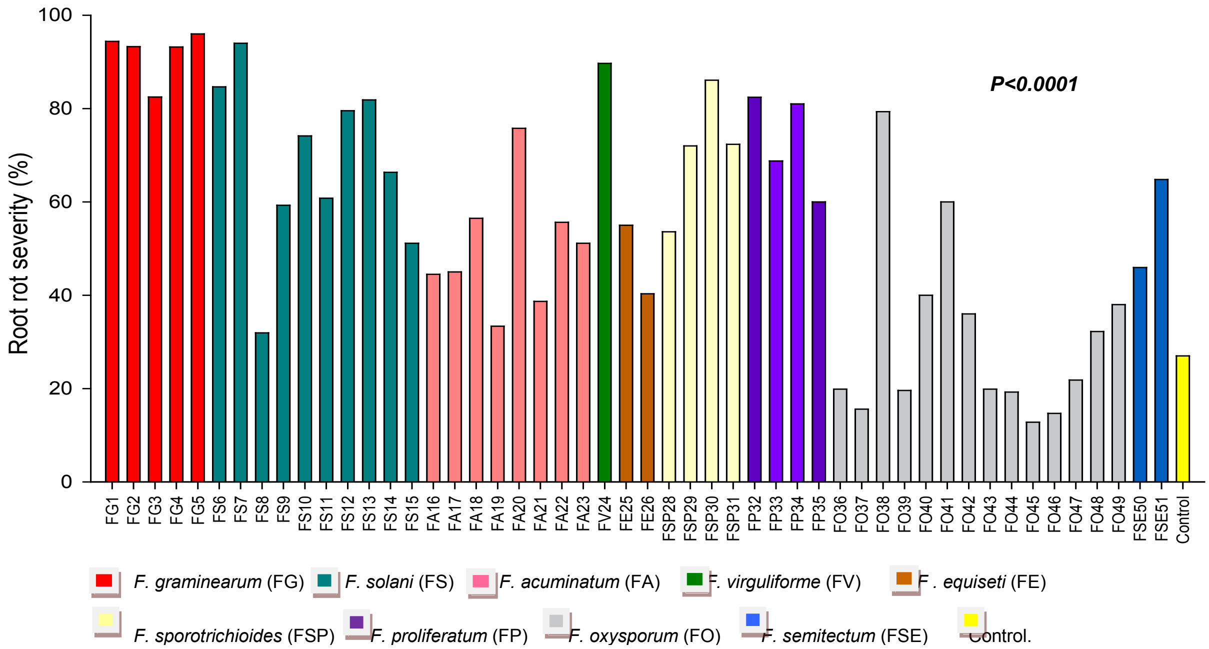

Figure 3. Root rot severity (%) on soybean roots inoculated with 49 *Fusarium* isolates at greenhouse conditions.

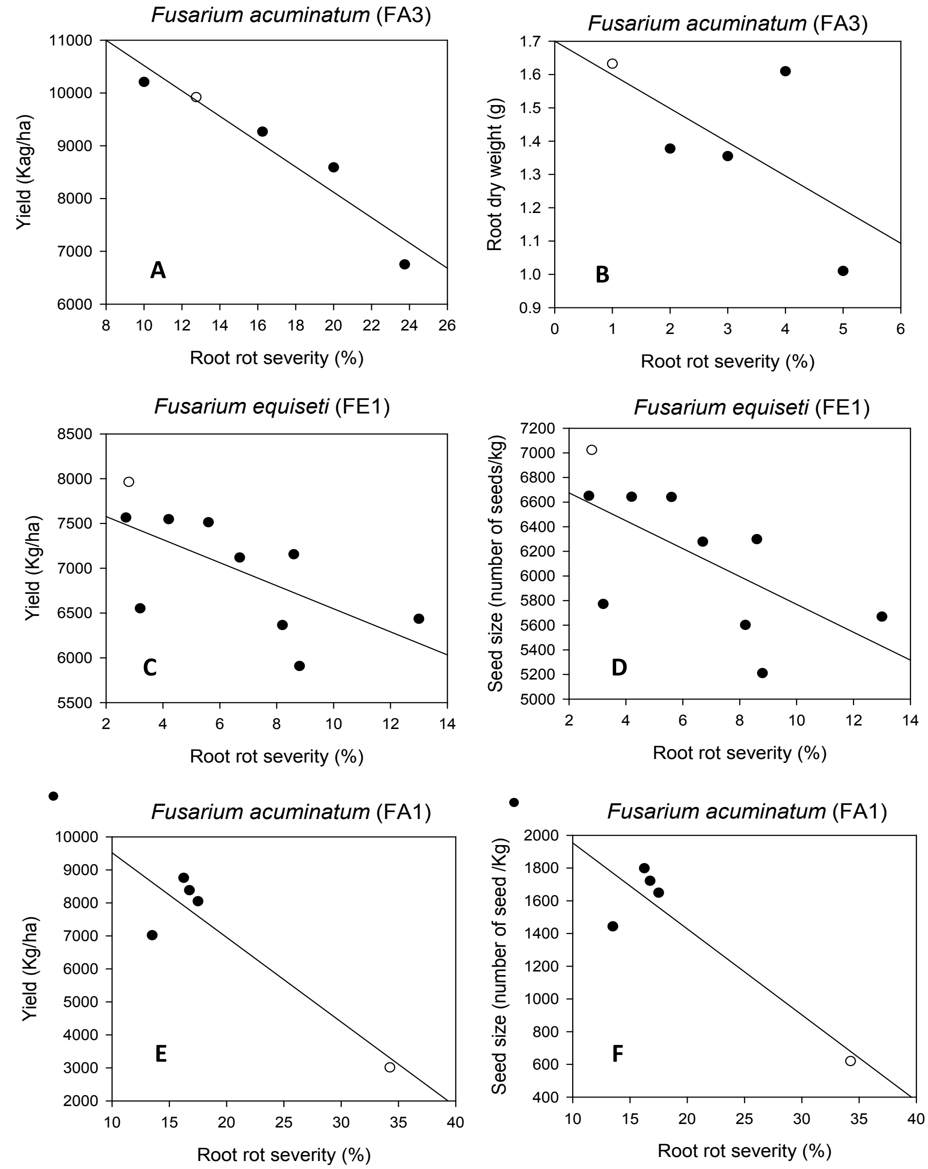

Figure 4. Relationship between root rot severity (%) with yield (kg/ha) and seed size in soybean plants inoculated with different *Fusarium* isolates in micrplots experiments at different locations in Iowa. A and B. *Fusarium acuminatum* (FA3) isolate at Horticulture Farm, Gilbert, IA, C and D *Fusarium equiseti* (FE1) at Hinds Farm, E and F. *Fusarium acuminatum* (FA1) at Horticulture Farm, Gilbert , IA.

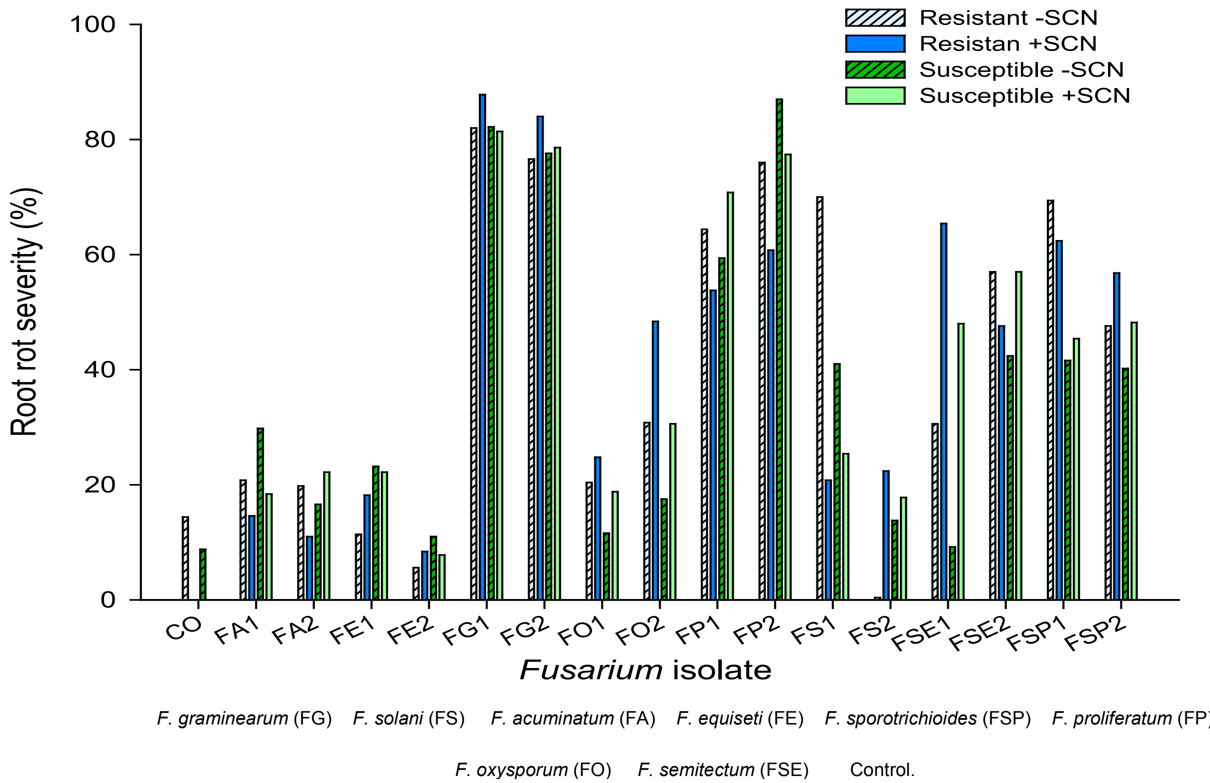

Figure 5. Root rot severity caused by *Fusarium* isolates alone or in combination with SCN, on resistant (Pioneer 92M54) and susceptible (Pioneer 92M91) soybeans.