

# Temporal dynamics of root and foliar severity of soybean sudden death syndrome at different inoculum densities

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**Abstract** Temporal dynamics of soybean sudden death syndrome (SDS) root and foliar disease severity were studied in growth chamber experiments on susceptible plants exposed to different inoculum densities (0,  $10^0$ ,  $10^1$ ,  $10^2$ , and  $10^3$  conidia  $g^{-1}$  soil) of *Fusarium virguliforme*. The monomolecular model provided the best fit to describe the progress of root and foliar disease severity over time. Disease severity and area under disease progress curve (AUDPC) both increased in response to increasing inoculum density ( $P < 0.01$ ), particularly for foliar symptoms. Rate of disease progress increased as inoculum densities increased for both root and foliar disease severities. The incubation period for root and foliar disease severity ranged from 9 to 18 and 15 to 25 days, respectively. Significant differences in root rot severity were most easily detected during the early stages of infection, whereas root rot and foliar severities were only weakly correlated when both were assessed simultaneously at later stages of disease development. Root rot severity assessments performed 15 to 20 days after inoculation (DAI) were most highly correlated ( $r > 0.9$ ,  $P < 0.01$ ) with foliar disease severity assessments performed 30 to 50 DAI. Root biomass was reduced by up to 67% at the three highest inoculum

densities, indicating the aggressiveness that *F. virguliforme* possesses as a root rot pathogen on soybeans.

**Keywords** Soil ecology · Mycology · Epidemiology · Disease progress

## Introduction

Sudden death syndrome (SDS) of soybean (*Glycine max* (L) Merrill) is a root rot and leaf scorch disease caused by *Fusarium virguliforme* (O'Donnell & T. Aoki), formerly *Fusarium solani* f. sp. *glycines* (Aoki et al. 2003). SDS is currently ranked among the top ten diseases that limit soybean yield in the North Central US (Wrather and Koenning 2009). Toxins produced by the pathogen in infected roots are translocated to the leaves, resulting in interveinal leaf scorch symptoms that are characteristic of the disease (Jin et al. 1996). However, the relationship between the severity of foliar symptoms and root rot severity is not well understood (Gray and Achenbach 1996; Hartman et al. 1997; Luo et al. 2000; Luo et al. 1999; Roy et al., 1997). Most research studies and resistance screening protocols have primarily focused on assessing the foliar phase of this disease (Hartman et al. 1997; Njiti et al. 2001; Rupe et al. 1991). As a result, relatively little is known about the root rot phase and its association with foliar symptoms (Gao et al. 2006; Gray and Achenbach 1996; Luo et al. 1999; Navi and Yang 2008; Njiti et al. 1997a, Roy et al. 1997). Several researchers have

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reported weak associations between root rot and foliar disease severities in studies comparing inoculum densities (Gao et al. 2006; Gray and Achenbach 1996), aggressiveness of pathogen isolates (Rupe, 1989), or the effect of soil temperatures on disease development (Scherm and Yang 1996). Results from these studies suggest that assessments of foliar symptoms alone provide a poor indication of root colonization by this pathogen. Several researchers have stressed the importance of assessing root rot in addition to foliar symptoms when determining SDS severity (Njiti et al. 1997a, Roy et al. 1997).

A major challenge in SDS research is the lack of consistency in disease development within and among screening trials (Gray and Achenbach 1996; Hartman et al. 1997; Njiti et al. 1997b; Roy et al. 1997). A primary factor that may be responsible for this variability in SDS symptom expression is variation in inoculum density in soil (Njiti et al. 1997b, 2001). In general, as inoculum density in soil increases, SDS foliar severity also increases (Gray and Achenbach 1996; Njiti et al. 2001), and positive associations have been reported between foliar disease severity and population density of *F. virguliforme* in field soil (Rupe et al. 1997; Scherm et al. 1998). However, the relationship between inoculum density and root rot severity is not clear (Gao et al. 2006; Gray and Achenbach 1996). Additionally, there is limited information concerning the effects of SDS on root growth (Gray et al. 1999; Gao et al. 2006).

Quantitative information concerning the relationship between SDS root and foliar disease severities over time at different inoculum densities may clarify the epidemiology of the disease and improve screening assays for resistance (Roy et al. 1997). The objectives of this study were to i) compare the temporal dynamics of SDS root and foliar disease severity at different inoculum densities and ii) quantify the impact of infection on root growth at different inoculum densities.

## Materials and methods

Susceptible soybean cultivar AG2403 (Monsanto Co., St. Louis, MO) was used in this study. Seeds were surface disinfested in 0.5% sodium hypochlorite for 2 min, followed by rinsing twice in sterile deionized water (SDW). The seeds were then placed in a tray

lined with moist sterile germination paper and incubated in the dark at 24°C until rootlets were approximately 1-cm long (approximately 4–5 days).

A single-spore isolate of *F. virguliforme*, obtained in 2006 from an infected plant from a field in Nevada, IA, was used. The isolate was cultured on carnation leaf agar and stored on potato dextrose agar (PDA) (Difco Industries, Detroit, MI) at 24°C. Prior to inoculation, the isolate was grown on PDA at room temperature (24±2°C) in darkness for 12 days. Conidia were dislodged from cultures by flooding with SDW, and the resulting suspension was filtered through three layers of sterile cheese cloth. The spore concentration was quantified and the suspension was diluted with SDW to obtain the following four conidial concentrations: 10<sup>1</sup>, 10<sup>2</sup>, 10<sup>3</sup> and 10<sup>4</sup> conidia ml<sup>-1</sup>. Sterile water was used as a control.

A two-way factorial, randomized complete block design was used, with five blocks, five inoculum density treatments, and six (destructive) sampling times. Pots were randomly assigned to each inoculum density and sampling time combination within each block. An experimental unit consisted of a pot containing three soybean plants, and there were five replicate pots (one per block) per inoculum density treatment for each sampling time. The experiment was conducted twice.

For inoculation, a mixture of pasteurized soil and sand (1:1 vol:vol) was amended with 1.5% (wt/wt) sterilized cornmeal to facilitate uniform infestation of the soil mixture by the pathogen. To obtain initial inoculum densities of 0, 10<sup>0</sup>, 10<sup>1</sup>, 10<sup>2</sup>, and 10<sup>3</sup> conidia g<sup>-1</sup> soil, the soil mixture was infested with the *F. virguliforme* conidial suspensions (100 ml kg<sup>-1</sup> soil) described above, or water. The soil for each inoculum density treatment was thoroughly mixed by hand, and immediately used to fill 12-cm-diameter plastic pots. Three pre-germinated seeds were then transplanted into each pot at a depth of 1 cm. Potted plants were incubated in a growth chamber at 17°C for 7 days to promote root infection, followed by 24°C for 43 days in the same growth chamber to promote foliar symptoms (Scherm and Yang 1996). The duration of the experiment was for 50 days after inoculation (DAI), at which time the soybean plants were at the V6 growth stage (Fehr et al. 1971). The photoperiod was 14 h light/10 h dark using both incandescent and fluorescent lights. Each pot was watered daily with approximately 100 ml of tap water using a hose.

Root rot and foliar disease severities were assessed visually at the following six destructive sampling times: 9, 15, 20, 30, 40 and 50 DAI. Foliar disease severity was estimated visually as the percentage of the total leaf area exhibiting chlorosis and necrosis. After washing roots under running tap water, root rot severity was assessed visually as the percentage of total root area exhibiting brown or black discoloration. Roots sampled at 20, 30, 40, and 50 DAI were dried at 89°C for 24 h and weighed to determine dry weight.

To quantify the isolation frequency of *F. virguliforme* from roots, two 1-cm-long root pieces (one piece from necrotic tissue and one from apparently healthy root tissue) were arbitrarily selected from each of the three soybean plants per pot at each of the six sampling times. Root pieces were then plated onto 9-cm-diameter petri dishes containing modified Nash and Snyder's medium (Cho et al. 2001), and cultures were incubated for 7 to 10 days in the dark at 24±2°C. The number of root pieces with blue, purple or white-coloured colonies typical of *F. virguliforme*, were counted 5 to 10 days after incubation. Fungal cultures were observed under the microscope for confirmation of *F. virguliforme* identity based on cultural characteristics and morphology of the macroconidia. Polymerase chain reactions (PCR) were also used to verify species identity of a subset of 30 cultures obtained 30 DAI (Li and Hartman 2003).

The area under the disease progress curve (AUDPC) and the area under the pathogen curve (AUPC) were calculated for each inoculum density using the trapezoidal integration method (Campbell and Madden 1990). The AUDPC was calculated based on progress curves for root rot severity and foliar severity, and AUPC was calculated based on progress curves for frequency of *F. virguliforme* isolations from roots. A mixed model analysis of variance was performed using the PROC MIXED procedure of SAS (SAS Institute, Cary, NC), using experimental run and the interaction between run and treatment as random factors, and treatment as a fixed factor. Pearson correlation coefficients ( $r$ ) were calculated to test the strength of the associations between root rot and foliar disease severity using treatment means within and among sampling times (Gomez and Gomez, 1984).

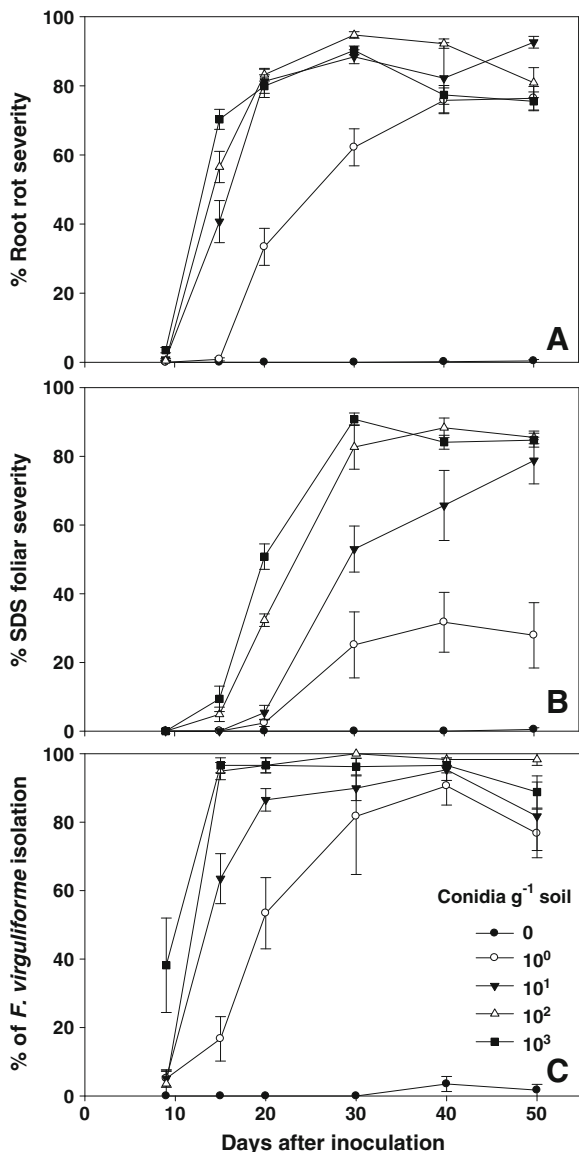
To characterize and compare the dynamics of root and foliar disease severity as affected by increasing

inoculum densities, the monomolecular population growth curve model ( $\ln[1/(1-y)] = \ln[1/(1-y_0)] + rt$ ) (Campbell and Madden 1990) was fitted to disease progress curves, where  $y_0$  is initial disease severity,  $y$  is disease severity at time  $t$ , and  $r$  is the rate of disease progress (slope of regression). Temporal population growth curves were modelled from the time disease symptoms were first detected until 40 DAI for all inoculum levels, except the highest inoculum density treatment for which disease progress was modeled until 30 DAI (disease severity was at  $y_{max}$  30 DAI for this treatment). Model fit was evaluated according to the significance of the  $F$ -statistic ( $P$  value) for the overall model, the coefficient of determination ( $R^2$ ), standard error for the estimate for  $y$  ( $SEEy$ ), and the normality of the residuals (assessed using the Shapiro-Wilk test) (Nutter, 1997). Linear regression analysis was conducted on data transformed using the monomolecular model and the slope values were used as a measure of the rate of disease progress. Incubation period was operationally defined as the number of days between inoculation and the time when 50% of plants showed symptoms within a treatment. Linear regressions were performed to quantify the relationships between root dry weight and sampling time (from 20 to 50 DAI), and the slope values were used as a measure of the rates of dry weight accumulation. Differences among rates of disease progress and root dry weight accumulation were tested using a mixed-model ANOVA, considering inoculum treatment as a fixed factor, and experimental run and interactions with run as random factors.

## Results

### Root rot and foliar disease severity

Root rot and foliar disease severity increased with increasing inoculum density, but the effect of inoculum density on disease severity was more pronounced on leaves than on roots (Fig. 1). The greatest differences in root rot severity among inoculum densities were observed 15 DAI, whereas root rot severities were similar for the three highest inoculum densities beginning 20 DAI (Fig. 1a). Foliar disease severity differed among inoculum densities across almost all sampling times 20 to 50 DAI (Fig. 1b). A decrease in



**Fig. 1** Disease progress curves for sudden death syndrome (SDS) root rot severity (a), foliar severity (b) and isolation frequency of *Fusarium virguliforme* from roots (c) of soybean plants (cv. AG2403) inoculated with five different conidial densities. Plants were incubated in a growth chamber at 17°C for 7 days, followed by 42 days at 24°C. Bars represent the standard error of the mean ( $n=10$ )

both root rot and foliar disease severities was observed between 30 and 40 DAI for the highest inoculum density, due to the production of new, apparently-healthy roots and leaves. Root rot severity and isolation frequency of *F. virguliforme* from roots had similar progress curves, with the greatest difference in *F. virguliforme* recovery among inoculum

densities occurring 15 DAI (Fig. 1c). Results of PCR tests confirmed the identity of 93% of the cultures tested as *F. virguliforme*. No disease developed on non-inoculated control plants (Fig. 1).

#### Relationship among root rot and foliar disease severity

Correlations between root rot and foliar disease severities varied greatly depending on time of assessment (Table 1). Correlation coefficients ( $r$ ) and corresponding  $P$  values indicated that root rot severity assessments conducted 15, 20, and 30 DAI were correlated ( $r \geq 0.88$ ,  $P < 0.05$ ) with foliar disease severity assessed 30 to 50 DAI, and with foliar AUDPC (Table 1), with the strongest correlations ( $r \geq 0.96$ ,  $P < 0.01$ ) observed when roots were assessed 15 and 20 DAI. Root rot severity assessed 40 and 50 DAI was not correlated with foliar disease severity assessments or foliar AUDPC. Frequency of *F. virguliforme* isolation from roots assessed 15 and 20 DAI was strongly correlated ( $r \geq 0.96$ ,  $P < 0.01$ ) with foliar disease severity assessed 30 to 50 DAI, but isolation frequency on roots assessed 40 and 50 DAI were not correlated with foliar assessments. Frequency of *F. virguliforme* isolation from roots assessed 15 and 20 DAI was also highly correlated ( $r \geq 0.94$ ,  $P < 0.01$ ) with foliar AUDPC.

#### Analysis of disease progress curves

Coefficients of determination ( $R^2$ ) for fit by the monomolecular model to disease progress ranged from 47 to 88% for root rot severity, and from 64 to 82% for foliar disease severity (Table 2). Analysis of residuals based on the Shapiro-Wilk test indicated normality. As inoculum density increased, rates of disease progress also increased for both root ( $P=0.004$ ) and foliar ( $P=0.003$ ) disease severity assessments performed up to 40 DAI (Table 2). The time to 50% severity occurred later in response to increasing inoculum densities on both roots and leaves. Root and foliar AUDPC and AUPC both increased ( $P \leq 0.007$ ) as inoculum density increased, but differences among the three highest inoculum densities were more evident in leaves than on roots (Table 3). Incubation periods were shorter for root rot severity (9 to 18 days) than for foliar severity (15 to 25 days), and were shortest at the highest inoculum densities (Table 3).

**Table 1** Pearson correlation coefficients (r) for the associations among foliar disease severity, foliar disease severity AUDPC, root rot disease severity, and frequency (%) of *Fusarium*

*virguliforme* isolation from roots, on soybeans (cv. Ag2403) exposed to five inoculum densities and assessed for sudden death syndrome symptoms at five assessment times

	Foliar severity (%) and foliar severity AUDPC					
	15 DAI	20 DAI	30 DAI	40 DAI	50 DAI	AUDPC
Root rot severity (%)						
9 DAI	0.86	0.83	0.70	0.59	0.58	0.69
15 DAI	0.84	0.88*	0.96**	0.93*	0.93*	0.96**
20 DAI	0.57	0.66	0.92*	0.97**	0.99**	0.93*
30 DAI	0.52	0.61	0.88*	0.94*	0.94*	0.89*
40 DAI	0.38	0.47	0.77	0.85	0.83	0.78
50 DAI	0.27	0.36	0.70	0.79	0.81	0.71
Frequency (%) of <i>F. virguliforme</i> isolation						
9 DAI	0.86	0.81	0.64	0.51	0.49	0.63
15 DAI	0.79	0.85	0.99**	0.98**	0.96**	0.99**
20 DAI	0.61	0.69	0.93*	0.97**	0.97**	0.94*
30 DAI	0.48	0.56	0.83	0.88*	0.86	0.83
40 DAI	0.41	0.46	0.77	0.84	0.82	0.78
50 DAI	0.49	0.57	0.83	0.89*	0.86	0.84

Data of two runs of the experiment were pooled ( $n=5$ )

\*\* $P \leq 0.01$ , \* $P \leq 0.05$

Root biomass

in inoculated plants was 37 to 73% lower compared to the non-inoculated control plants (Table 4). At the time of final assessment, root dry weight of plants inoculated with the three highest

Linear regression between DAI and root dry weight showed that the rate of dry weight accumulation

**Table 2** Summary of regression analysis parameters and statistics for linear transformations of data using the monomolecular population growth curve model ( $[\ln(1/(1-y))]$ ) fitted to

describe the progress of root rot severity (%) and foliar disease severity (%) of sudden death syndrome caused by *Fusarium virguliforme* on soybean (cv. AG2403)

	Inoculum density (conidia $g^{-1}$ soil)	Intercept	Slope	$R^2$	$P$ value	$SEE_y$	Residuals <sup>b</sup>
Root rot severity	$10^0$	-0.614	0.059	88	0.018	0.08	0.547
	$10^1$	-0.146	0.095	83	0.040	0.37	0.509
	$10^2$	-0.387	0.089	78	0.055	0.57	0.580
	$10^3$	0.286	0.110	47	0.243	0.73	0.320
$P = 0.004^a$							
Foliar severity	$10^0$	-0.269	0.025	64	0.151	0.188	0.519
	$10^1$	-0.67	0.081	80	0.050	0.176	0.560
	$10^2$	-1.09	0.100	82	0.039	0.330	0.487
	$10^3$	-0.789	0.176	73	0.072	0.50	0.410
$P = 0.003^a$							

<sup>a</sup>  $P$  values obtained from ANOVA ( $n=10$ ) to compare slopes (rate of disease progress) among treatments

<sup>b</sup> Statistical significance of the analysis of normality of the residuals based on the Shapiro-Wilk test. All  $P$  values were  $>0.05$ , indicating the residuals were normally distributed

For the  $10^3$  inoculum density treatment, the temporal analysis was done up to 30 DAI

**Table 3** Area under disease progress curve (AUDPC), incubation period, and area under the pathogen curve (AUPC) for soybean plants (cv. AG2403) inoculated with five inoculum densities of *Fusarium virguliforme*, causal agent of sudden death syndrome

Inoculum density (conidia g <sup>-1</sup> soil)	Root rot severity		Foliar severity		<i>F. virguliforme</i> isolation AUPC
	AUDPC	Incubation Period (days)	AUDPC	Incubation Period (days)	
0	0±0 <sup>a</sup>	–	0±0	–	33±22
10 <sup>0</sup>	1883±171	18	731±185	20	2357±285
10 <sup>1</sup>	2867±206	12	1548±192	25	3230±117
10 <sup>2</sup>	3159±45	9	2365±94	18	3703±47
10 <sup>3</sup>	3006±43	9	2552±49	15	3555±178
P value	0.0042 <sup>b</sup>	–	0.0030	–	0.0072

<sup>a</sup> mean ± standard error

<sup>b</sup> P value obtained from ANOVA ( $n=10$ ) to compare AUDPC and AUPC among treatments

inoculum levels was up 67% lower than control plants (Fig. 2).

## Discussion

In this study, the progress of SDS root rot and foliar disease severities over time was compared in soybeans grown in soils infested with a range of *F. virguliforme* inoculum levels. Few other SDS studies (Njiti et al. 1997a; Luo et al. 1999, 2001) have compared the relationship between progress of the root and foliar phases of the disease. Early assessments of root rot severity caused by *F. virguliforme* were found to be more highly correlated with assessments of SDS foliar severity than those performed in later phases of root rot development. Specifically, root

rot severity assessed 15 to 20 days after inoculation had the highest correlations when foliar disease severity was assessed 15 to 25 days after the root assessments. However, root rot and foliar severities were not as strongly correlated if disease assessments were conducted simultaneously at the end of the experimental period. These results are consistent with earlier SDS studies that reported weak correlations between root and foliar severities when both variables were assessed simultaneously at later stages of disease development in controlled environment (Gray and Achenbach 1996; Rupe 1989; Scherm and Yang 1996).

The weak, or lack of, correlation between root and foliar disease severities assessed at the same time may be explained by the pathogenesis of *F. virguliforme*. The fungus first infects the roots and must then colonize the xylem (Navi and Yang 2008) so that

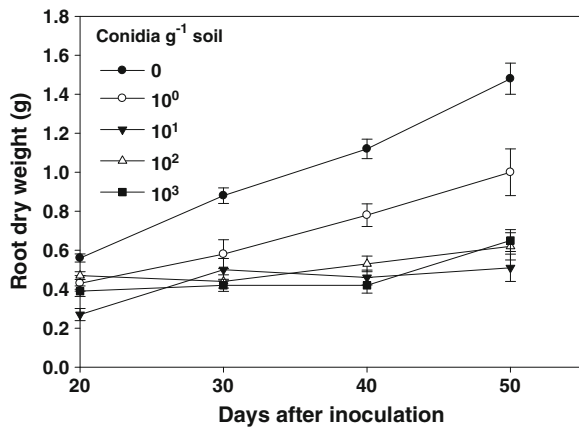
**Table 4** Parameter estimates and statistics obtained for the linear regression of root dry weight (g) over time on soybean plants (cv. AG2403) inoculated with five densities of *Fusarium virguliforme*

Inoculum density (conidia g <sup>-1</sup> soil)	Intercept	Slope	R <sup>2</sup>	P value	SE <sub>E</sub> <sup>a</sup>	Residuals <sup>b</sup>
0	-0.052	0.030	0.99	0.002	0.001	0.698
10 <sup>0</sup>	0.032	0.019	0.99	0.003	0.0006	0.422
10 <sup>1</sup>	0.187	0.007	0.63	0.200	0.006	0.195
10 <sup>2</sup>	0.334	0.005	0.70	0.159	0.002	0.582
10 <sup>3</sup>	0.185	0.008	0.70	0.162	0.006	0.68
		P<0.0001 <sup>c</sup>				

<sup>a</sup> Standard error of the estimate of y

<sup>b</sup> Statistical significance of the analysis of normality of the residuals based on the Shapiro-Wilk test. All P values were >0.05, indicating the residuals were normally distributed

<sup>c</sup> P value obtained from ANOVA ( $n=10$ ) for comparison of slopes (rate of dry weight accumulation) among treatments



**Fig. 2** Effect of five conidial densities of *Fusarium virguliforme* on root dry weight (g) of soybean plants (cv. AG2403) over time (a). Bars represent the standard error of the mean ( $n=10$ )

pathogen toxins can be translocated to the leaves, where they cause cell damage and consequent appearance of visible symptoms (Jin et al. 1996). This process causes a lag in the expression of leaf symptoms in relation to development of root rot. This process may be even more extended under field conditions as foliar symptoms typically appear at or after flowering (Roy et al. 1997). It is also possible that roots may outwardly exhibit severe root rot, yet pathogen colonization is limited to the cortex, thereby restricting upward movement of the toxin to the leaves (Navi and Yang 2008). The fact that isolation frequency of *F. virguliforme* from roots was similar among all inoculum density treatments at assessment times when foliar disease severity differed in this study, suggests that colonization of the roots may not be indicative of the amount of pathogen in the xylem. Weak correlations have been reported between a foliar disease index and root infection frequency in field-grown plants (Njiti et al. 1997a; Luo et al. 1999, 2001). Therefore, care must be taken when interpreting the significance of root rot severity or isolation frequency of *F. virguliforme* as measures of soybean resistance to SDS.

Host plant resistance and plant developmental stage may also affect the relationship between disease severity on roots and leaves (Kazi et al. 2008). Several quantitative trait loci (QTL) have been found to be associated with resistance to SDS root rot and foliar symptoms. Moreover, soybean cultivars may have resistance to one or both phases of the disease, depending on the combination of QTL in the plant

(Kazi et al. 2008; Njiti et al. 1997a). The interactions between host resistance, plant growth stage, and environment are also likely to quantitatively affect specific components of resistance, such as infection efficiency and incubation period, thereby affecting the relationship between root and foliar symptoms (Kazi et al. 2008; Njiti et al. 1997a).

Gray and Achenbach (1996) did not find clear differences in root rot severities as affected by a range of inoculum densities on both resistant and susceptible soybean cultivars. Although a single susceptible cultivar was used in the present study, the results suggest that the timing of root and foliar assessments for disease severity may also have played a role in the lack of strong associations between root and foliar severity in their study and others (Gray and Achenbach 1996; Scherm and Yang 1996). In the present study, the time to reach 50% root rot severity was delayed as inoculum density decreased, yet time to 80% root rot severity was not as affected by inoculum density (Fig. 1). Differences in root rot may, therefore, not be as evident at the conclusion of experiments, when root rot severity has become severe in all treatments.

The best fit of the monomolecular model to the root and foliar disease progress curves is consistent with other monocyclic diseases, whereby the rate of disease progress is fastest during the early stages of the epidemic and then progressively slows due to a decrease in the amount of apparently healthy plant tissue (Campbell and Madden 1990). Although inoculum densities in soil were not monitored over time, there was clearly an increase in root and foliar severity in response to increasing levels of initial inoculum in soil infested with *F. virguliforme*. The rates of disease progress were faster at the higher inoculum densities likely due to an increase in the number of infections per root, as supported by the increase in isolation frequency with respect to inoculum levels. The effect of inoculum density was particularly noticeable in the early stages of disease development, indicated by the shorter time it took to reach 50% severity.

In the present study, root infection by *F. virguliforme* caused up to 67% loss in root biomass indicating that this fungus is an aggressive root pathogen. Other researchers (Gao et al. 2006; Gray et al. 1999; Rupe 1989) have also reported that *F. virguliforme* can reduce root and shoot dry weight on soybeans, but this is the first study to measure the impact of *F. virguliforme* on root growth over time at

several inoculum densities. Roots of plants exposed to an initial density of 10 or more conidia per gram of soil almost stopped growing, but roots continued to grow at a constant rate in plants exposed to an initial density of one conidium per gram of soil. Root growth parameters were previously suggested as better indicators of root infection than root rot severity (Rupe 1989).

In summary, inoculum density was shown to affect the progress of root rot and foliar disease severities, but the impact of inoculum density on root rot severity was most detectable in the early stages of disease progress. Therefore, in greenhouse assays in which soybean plants are assessed during the vegetative growth stages, root rot assessments performed earlier than foliar disease assessments may be more informative than if root rot assessments are conducted after the pathogen has already extensively colonized the roots. This is particularly important for studies conducted under high inoculum densities, where the rate of root rot development is initially very fast. The fact that root biomass was greatly reduced by *F. virguliforme* suggests a need to investigate the impact of the root rot phase of SDS on yield losses, even when foliar symptoms are absent. Additional work with other soybean cultivars and in field trials is needed, however, to verify if similar associations between early root rot severity assessments and foliar assessments performed at later stages of disease development occur in field conditions.

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