Effects of Soil Conditions on Root Rot of Soybean Caused by *Fusarium graminearum*

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ABSTRACT

Fusarium graminearum is an important soybean pathogen that causes seedling disease, root rot, and pre- and postemergence damping-off. However, effects of soil conditions on the disease are not well understood. The objective of this greenhouse study was to determine the impacts of soil texture, pH, and soil moisture on seedling root rot symptoms and detrimental effects on seedling development caused by *F. graminearum*. *F. graminearum*-infested millet was added (10%, vol/vol) to soil with four different textures (sand, loamy sand, sandy loam, and loam). Soil moisture was maintained at saturation, field capacity or permanent wilting point at soil pH levels of 6 or 8. Seedlings were evaluated 4 weeks after planting for root rot, root length, root and shoot dry weights, leaf area, and *F. graminearum* colonization (by qPCR). There was a significant interaction between soil moisture and soil

texture for root rot assessed visually ($P < 0.0001$). Highest severity (67%) and amount of *F. graminearum* DNA were observed at pH 6 and permanent wilting point in sandy loam soils. Pot saturation resulted in the lowest levels of disease in sandy loam and loam soils (11.6 and 10.8%, respectively). Reductions in seedling growth parameters, including root length, foliar area, shoot and root dry weights, and root tips, relative to the noninfested control, were significantly greater in sandy loam soils. In contrast, there were no significant growth reductions in sand. This study showed that levels of root rot increased under moisture-limiting conditions, producing detrimental effects on plant development.

Keyword: mycology

Fusarium graminearum (synonym *Gibberella zeae* (Schwein.) Petch) is a major necrotrophic pathogen of soybean, causing pod blight, seed decay, damping-off, and crown and root rots in the United States, Canada, and other temperate regions (Martinelli et al. 2004; Sella et al. 2014; Xue et al. 2007). Soybean is often included in rotation with wheat, maize, and other cereal crops that are *F. graminearum* hosts; *F. graminearum* colonizes and overwinters on these crop residues providing a source of inoculum for subsequent seasons (Barros et al. 2014; Leslie et al. 1990; Windels et al. 1988). *F. graminearum* isolates that colonize wheat and maize have been found to be highly pathogenic on soybean (Broders et al. 2007). Xue et al. (2007) suggested that soybean and cereal crop rotations provide strong selection pressure for the development of highly aggressive *F. graminearum* isolates on all crops. Isolates of *F. graminearum* from soybean in the Central United States have been characterized as 15-ADON genotypes of *F. graminearum sensu stricto* (Ellis and Munkvold 2014). Other trichothecene genotypes have not been reported from soybean, to our knowledge.

There are reports of *F. graminearum* being highly prevalent and associated with soybean seedlings causing severe root rot in Iowa (Díaz Arias et al. 2013a, b). Seedling diseases caused by diverse fungi and Oomycetes generally are prevalent when soil temperatures are low and soil moisture is high. These conditions delay germination and emergence, increasing the probability of seed and seedling infection (Broders et al. 2007; Licht et al. 2013).

Disease severity for root pathogens is influenced by soil physiochemical conditions and biodiversity in soil microbial populations (Ghorbani et al. 2008). However, there is a lack of detailed knowledge about soil environment and soil conditions such as pH, soil texture, and soil moisture that influence disease development, incidence and severity of *Fusarium* root rot of soybean caused by *F. graminearum*.

Soil pH influences root rot severity by directly affecting soil microbial communities and the balance between pathogens and their specific antagonists (Hoper and Alabouvette 1996). Evidence suggests that lowering soil pH from 8 to 6 on *Fusarium*-suppressive soils drastically reduces the viability of *Pseudomonas* spp. and eliminates the suppressive effects on *Fusarium* wilt diseases (Scher and Baker 1980). Similarly, changes in soil pH indirectly affect nutrient availability to the pathogen and the plant host (Ghorbani et al. 2008).

Soybeans are sensitive to changes in soil pH; highest soybean yields in Iowa and Minnesota have been reported at soil pH levels between 6.2 and 7.0 (McLean and Brown 1984; Rogovska et al. 2007). Altered nutrition induced by soil acidity can increase or decrease the susceptibility of the plant host, affecting disease severity and incidence (Fang et al. 2012; Ghorbani et al. 2008). Under high soil pH, iron availability is reduced and pathogen establishment will depend on the ability to acquire and compete for iron. *F. graminearum* has two types of iron acquisition systems, secretion and uptake of iron-chelating molecules and production of cell wall iron reductases to reduce ferric iron to ferrous iron (Greenshields et al. 2007; Kosman 2003; Philpott 2006). Iron availability also affects soybean plants and can lead to iron deficiency chlorosis (IDC) in high pH soils (Rogovska et al. 2007). Soybean plants with IDC symptoms often display root rot symptoms, but the basis of this association is not clear.

Soil texture can also affect pathogen distribution in soil and plant resistance to disease. *Fusarium* wilts have been commonly associated with sandy soils (Dominguez et al. 1996; Toussoun 1975). For example, field observations have shown that *Fusarium*

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wilts caused by *F. oxysporum* are more severe in coarse sandy soils or loamy-textured soils, and clayey soils seem to be suppressive (Hoper and Alabouvette 1996; Scher and Baker 1980; Yuen et al. 1983). However, associations between Fusarium root rot of soybean caused by *F. graminearum* and soil texture components have not been documented. Soil texture and type of clay have been correlated to disease incidence and severity in a diverse number of Fusarium wilts on muskmelon, banana, flax, barley, and tobacco (Alabouvette et al. 1979; Dominguez et al. 1996; Hoper et al. 1995; Strunnikova et al. 2015; Stutz et al. 1989; Toussoun 1975).

Bacteria actively interact with soil clay minerals due to the high cationic exchange capacity (CEC), large surface area and variable negative charge (Hoper and Alabouvette 1996); these interactions consist mainly of buffering soil pH, providing available nutrients that can be rapidly used. Addition of clay minerals into the soil reduces the availability of iron to fungal pathogens, promoting bacterial growth (Hoper et al. 1995). High clay CEC stimulates the metabolism of bacteria (Stotzky and Rem 1966). In contrast, silt-sand minerals are considered less active (Robert and Chenu 1992).

Soil texture is related to different physical soil characteristics such as the distribution of pore sizes, soil structure, and soil water potential; these soil attributes determine the habitable space and distribution of bacteria and fungi (Hoper and Alabouvette 1996). Soil texture and soil water holding potential also contribute to host growth, root architecture, and pathogen resistance (Ali and Ghaffar 1991; Krizek et al. 1985; Lynch and Vanbeem 1993).

Depending on the pathosystem, low and high soil water potential may have different effects on root rot. For example, in *F. oxysporum*-infested soils near saturation, soybean root rot severity increased, but only at low soil temperatures (French 1963). Field observations indicate that three or 7-day duration of flooding at soybean emergence increase incidence and severity of *Pythium* spp., whereas at the same time *Fusarium* spp. tend to be less frequently associated with seedling infection under flooded conditions (Kirkpatrick et al. 2006). On the other hand, low water potentials may predispose the host to infection and favor diseases such as dry rot of beans caused by *F. solani* and seedling blight of cereals caused by *F. roseum* (Ghorbani et al. 2008; Hoper and Alabouvette 1996). Fusarium root rots of sweet potatoes, peas, and beans are also increased by dry soil conditions (Cook and Papendick 1972). In addition, several Fusarium wilt diseases are favored by dry soil conditions; Fusarium wilt of chickpea caused by *F. oxysporum* f. sp. *ciceris* (Landa et al. 2006; Navas-Cortes et al. 2007), seedling blight of clover caused by *F. roseum* (Graham et al. 1957), and root and stem rot of peas and chickpeas caused by *F. solani* f. sp. *pisi* (Bhatti and Kraft 1992).

The objectives of this research were to measure the effects of important soil variables on the development of root rot of soybean caused by *F. graminearum* by studying (i) the effects of soil pH and soil water content interaction on root rot, (ii) the effects of artificial soil textures and its interaction with soil pH and soil water content on root rot, and (iii) the possible detrimental effects on soybean seedling growth parameters under various edaphic conditions on *F. graminearum*-infested soils.

MATERIALS AND METHODS

Inoculum production. *F. graminearum* isolate FG5 was collected from symptomatic soybean roots in Iowa in 2007, single-spored, identified to species microscopically and by DNA sequencing (Díaz Arias et al. 2013b), and maintained on dry silica gel beds at 4°C until use. Aggressiveness and pathogenicity of the FG5 isolate was tested on soybean cultivar Asgrow 2403 (SDS susceptible) (Monsanto Co., MO) under greenhouse conditions (Díaz Arias et al. 2013a).

Millet seeds were soaked for 24 h in water, drained and autoclaved at 121°C for 1 h in autoclave bags with a microporous filter patch on two consecutive days. Isolate FG5 was grown on

potato dextrose agar (PDA) 39 g/liter (Difco, Becton, Dickinson and Co, MD) for 14 days in an incubator at 25°C with a 12-h photoperiod, to promote conidial formation. Plugs (~1 cm²) from cultures of FG5 were added to the sterile millet in a biosafety cabinet and then sealed with a rubber band. Bags were then placed in an incubator (Hoffman Manufacturing Inc., OR) for 8 days, at 65% relative humidity with a 12 h photoperiod, and mixed by hand every day. FG5-infested millet was mixed with soil at 10% concentration by volume.

Artificial soil textures. Four artificial soil textures (sand, loamy sand, sandy loam, and loam) were created by mixing sand and silt loam in different proportions by volume (Table 1). Resulting textural classes on the artificial soils were analyzed for particle size distribution following the protocol by the National Soil Survey Center (1996). Soils were pasteurized in a soil steamer with a steam generator SG670 and steam aerator SA700 (Lindig Manufacturing, Co., MN) for 1.5 h, dried on a plastic sheet on a greenhouse bench for 4 days and kept in closed plastic containers until use.

Soil pH. Initial soil pH for each soil textural class was measured by the electrometric standard method, using a 1:1 soil/water ratio (Watson and Brown 1997). Ten milliliters of distilled water was added to 10 g of soil in a 50-ml falcon tube, shaken for 10 min and settled for 5 min, placing the pH meter electrode in the slurry. After the initial pH measurements, soil pH was adjusted to 6 or 8 by gradually adding Al₂(SO₄)₃ (Bonide Products, Inc., NY) and CaCO₃ (Fisher, IL) to the soil, respectively. Soils were then wet, dried, and mixed twice a day for 2 weeks to allow the soil to react with the chemical compounds. Soil pH measurements were performed periodically until the soil remained at constant pH equilibrium (Islam et al. 2004).

Soil moisture content. Three levels of soil moisture including pot saturation (PS), field capacity (FC), and permanent wilting point (PWP) were set for each soil texture, derived from the volumetric water content (θ_v) points for different textural classes described by Rowell (1994) and Brady and Weil (2004) (Table 2). For each soil textural class, 230 ml of dried soil was weighed in a 350-ml Styrofoam cup, and a calculation of the volume of water needed for each type of soil texture was performed by using the following formula: $V_w = V_s \times \theta_v$, where V_w is the volume of water contained in a soil, V_s is the total volume of soil, and θ_v is the soil volumetric water content point. The weight of 230 ml of soil for each soil textural class and the weight of the water needed to reach the desired water content point were added to have a final weight per cup that was subsequently maintained by adding water once a day.

Soil infestation and experimental design. After 2 weeks of incubation, inoculum was mixed with soil at 10% concentration by volume. Noninfested control treatments contained 10% autoclaved sterile millet. Seeds of soybean cultivar Jack were surface disinfested in 0.5% NaOCl for 2 min, rinsed twice for 2 min in sterilized distilled water, and dried for 20 min in a laminar flow hood before planting. Three seeds per cup were planted into the infested or noninfested soil mix and watered daily for 2 weeks. Soil moisture levels PS, FC, and PWP were imposed 2 weeks after planting and maintained for 2 weeks. Four weeks after planting, seedlings were visually rated for root rot severity from 0 to 100% of roots with brown or black discoloration, roots were scanned using a flatbed scanner (EPSON Expression, 10000XL, Epson America, Inc.), and root images were analyzed using WinRhizo software 2008 (Regent Instruments Inc., QC, Canada) to obtain estimates of root length, surface area, root volume, root tips, and forks on each individual plant. In addition, foliar area was estimated on individual plants using Assess 2.0 (The American Phytopathological Society [APS], St. Paul, MN). Shoot and root dry weights were measured on each individual plant after oven drying at 80°C for 24 h. Percentage reduction of seedling growth parameters was calculated measuring root length, foliar area, root dry weight, shoot dry weight, root volume, and root tips on each plant from the soil infested treatments and then dividing by the average measure of the noninfested control

on each soil texture, soil moisture, and soil pH combination treatment and multiplying by 100. The experiment was organized in a split-plot design with soil moisture (PS, FC, and PWP) as the main-plot factor, and soil texture (sand, loamy sand, sandy loam, and loam), soil pH (6 or 8), and *F. graminearum*-infested or noninfested soils as subplot factors. One cup was considered as an experimental unit with three seeds or subsamples in each cup. The experiment had eight replications per treatment combination and was conducted twice.

DNA extractions. DNA was isolated from root samples obtained from plants growing in the greenhouse at soil pH 6 after 30 days. Twenty-four roots (biological replicates) per treatment combination were arbitrarily selected, washed under tap water, surface-disinfested in 0.5% NaOCl for 2 min, rinsed three times in sterilized distilled water, air-dried, lyophilized for 2 days, ground in liquid nitrogen with a sterile pestle, and stored at room temperature until use. Root DNA was extracted using a modification of the CTAB (2%) procedure by Doyle and Doyle (1987), and purified in the KingFisher Flex Magnetic Particle Processor System using the MagMAX Plant DNA isolation kit A32549 (Applied Biosystems, CA).

For fungal DNA extraction, the FG5 isolate was grown on potato dextrose broth in 125-ml plastic flask for 5 days in an incubator at 25°C while shaken at 125 rpm. The fungal tissue and broth were strained through cheesecloth, lyophilized for 1 day, ground in liquid nitrogen, placed in a 1.5-ml tube, and frozen at -80°C until use. Genomic DNA of mycelia were extracted by the CTAB (2%) method.

The concentration of the DNA (ng per µl) and ratios of absorbance (A_{260}/A_{280} and A_{260}/A_{230}) were quantified spectrophotometrically in a NanoDrop ND-1000 (NanoDrop Technologies, DE).

qPCR assay. To quantify the *F. graminearum* DNA concentration in the soybean roots a qPCR IDT PrimeTime gene expression master mix assay was designed to amplify the translation elongation (*tef1α*) gene of *F. graminearum*. The PCR amplifications were performed in total volumes of 15 µl using a 96-well microwell plates, each reaction contained: 13 µl of master mix, 1 µl (10 µmol/liter) of forward primer (5'-CTGCCCTCTTCCCACA-3'), 1 µl (10 µmol/liter) of reverse primer (5'-AGAACCCAGGCG TACTTG-3'), and 0.375 µl (10 µmol/liter) of probe (5'-FAM CCACCTGTC/ZEN/ATTAGGAAGC-3'). Amplifications were performed using a Bio-Rad CFX384 C1000 Touch thermal cycler (Bio-Rad Laboratories, CA), in which thermal cycling conditions consisted of one cycle of 95°C for 3 min, 40 cycles of 95°C for 15 s followed by one cycle of 60°C for 1 min.

DNA standard curves were obtained from 10-fold serial dilutions of fungal DNA (FG5 isolate), using three replicates of six concentrations (1:1, 1:10, 1:10², 1:10³, 1:10⁴, and 1:10⁵) in sterile ultrapure water. The cycle threshold (Ct) values obtained from each dilution series were used to calculate the value of the target DNA. The correlations between the Ct values and the DNA quantities was high for *F. graminearum* ($R^2 = 0.9951$). Both ultrapure water and roots from the noninfested pots were used as negative controls for the qPCR reactions.

Data analysis. Visual root rot severity data (%) was arcsine transformed. Root length, surface area, root volume, root tips, forks, foliar area, and shoot and root dry weight were square root

transformed to meet the assumptions of analysis of variance. In addition, normal distribution of the residuals were tested for all the transformed data under the PROC UNIVARIATE procedure of SAS version 9.3 (SAS Institute Inc., Cary, CNC). Separate analysis of the two experimental replications showed similar trends, so data were combined for subsequent analyses. Analysis of variance was performed on the transformed data using PROC GLIMMIX in which the main-plot effect, soil moisture, was tested against the whole-plot variation error, and subplot effects were tested against the residual variability. Mean separation analyses on the transformed data were performed using a Fisher's protected least significant difference (LSD) test at $P = 0.05$. In addition, correlations using the PROC CORR statement were performed to compare the average of root rot severity (%) and fungal DNA quantity in the root tissue of plants growing on infested soil at pH 6.

RESULTS

Root rot severity. The analysis of variance indicated a significant effect for the main factors soil moisture content (θ_v), soil pH, and soil texture, as well as the interaction between θ_v and soil texture ($P < 0.0001$) for the visual evaluation of root rot (Table 3). These significant interactions indicate that the level of disease varied across the soil textures and the effect of soil moisture content varied among soil textures, primarily because soil moisture content did not affect root rot in sand.

Soil pH significantly influenced disease severity. Soil at pH 6 showed more disease in all treatment combinations compared with pH 8 (Fig. 1). Overall, across soil textures, the highest level of root rot was observed at pH 6 and PWP (55.3%); and the lowest level of disease was observed at pH 8 and PS (15.4%) (Fig. 1A).

The effects of soil moisture and soil texture on root rot severity displayed similar trends for pH 6 and 8. Root rot severity increased in the finer textured soils at PWP. Conversely, disease severity decreased in the finer textured soils at PS; therefore, PWP and PS demonstrated inverse trends on root rot severity. Disease severity at FC remained between the highest and lowest disease levels in each soil texture. In addition, there were no significant differences in the levels of disease across the soil moisture levels in the sand treatment (Fig. 2).

The highest levels of disease occurred in loamy sand and sandy loam soils at PWP at both pH levels (Fig. 2). At soil pH 6, the highest levels of disease were 52.6 and 67%, in loamy sand and sandy loam soils, respectively, whereas at pH 8, they were 51.7 and 49.8%. In contrast, the lowest levels of root rot were found in sandy loam and loam soils (10.2 and 9.7%, respectively) at PS and soil pH 8

TABLE 2. Values of volumetric water content of soils of varying soil textures^a

Soil textures	Soil volumetric water content (θ_v)		
	PS	FC	PWP
Sand	0.13	0.09	0.07
Loamy sand	0.16	0.10	0.08
Sandy loam	0.25	0.17	0.09
Loam	0.28	0.21	0.11

^a Soil moisture levels: PS = pot saturation, FC = field capacity, and PWP = permanent wilting point.

TABLE 1. Artificial soil textural classes generated by sand and silt loam mix^a

Soil mix by volume		Particle size distribution analysis				
Sand (%)	Silt loam (%)	Textural class	Sand (%)	Silt (%)	Fine silt (%)	Clay (%)
90	10	Sand	95.90	1.49	1.94	0.67
75	25	Loamy sand	87.14	4.62	3.96	4.28
25	75	Sandy loam	61.94	13.67	11.84	12.55
10	90	Loam	42.10	19.05	19.88	18.97

^a Particle size distribution analyses for the artificial soil textural classes obtained using the pipette method.

(Fig. 2B), followed by sandy loam and loam soils (21.5 and 19.9%, respectively) at PS and soil pH 6 (Fig. 2A).

Effects on seedling growth variables. Comparisons between *F. graminearum*-infested and noninfested treatments revealed detrimental effects on shoot and root morphological characteristics, especially in loamy sand and sandy loam soils (Table 4). Plants growing in *F. graminearum*-infested soils had significantly shorter roots compared with the noninfested control in loamy sand and sandy loam soil textures at all soil moisture levels. In contrast, root length was not affected by *F. graminearum* when plants were grown in sand (Fig. 3A).

There were significant detrimental effects on foliar area for *F. graminearum*-infested sandy loam soil at all soil moisture content levels, and loam soil at PS. Conversely, foliar area was significantly higher in the infested treatments compared with noninfested treatments in sandy soils at FC and PWP (Fig. 3B).

TABLE 3. Analysis of variance indicating the effects of soil moisture content, soil pH, and soil texture on visual root rot severity (%), following soil infestation with *Fusarium graminearum*^a

Source	df	F value	P > F
Soil moisture content (θ_v)	2	73.09	<0.0001
Soil pH (pH)	1	50.42	<0.0001
$\theta_v \times \text{pH}$	2	0.57	0.5675
Soil texture (ST)	3	14.51	<0.0001
$\theta_v \times \text{ST}$	6	16.66	<0.0001
pH $\times$ ST	3	1.40	0.2406
$\theta_v \times \text{pH} \times \text{ST}$	6	0.77	0.5956

^a Analysis of variance was performed on the *F. graminearum*-infested plants only. Visual root rot disease data (%) were arcsine transformed. The experiment consisted of eight reps in a split-plot design, with soil moisture content as the main-plot factor, and soil pH and soil texture as the subplot factors.

Root and shoot dry weight were negatively affected by *F. graminearum* in sandy loam soil at PS and FC. However, there were no detrimental effects on root dry weight for the plants growing in infested sand (Fig. 3C and D).

Root volume was significantly affected by *F. graminearum*. There were significant differences in root volume between infested and noninfested plants for all soil textures and soil moisture treatments except for sand (Fig. 3E). Number of root tips was significantly affected by *F. graminearum* root infection in sandy loam and loam soils but no adverse effects were observed in sand or loamy sand soils (Fig. 3F).

The greatest reductions in the root and shoot morphological characteristics occurred in *F. graminearum*-infested sandy loam soils (Fig. 4). The greatest reductions in root length were observed in sandy loam soils in all levels of soil moisture (21 to 26%) followed by loam soil at FC (18.6%) (Fig. 4A). Foliar area was significantly reduced in sandy loam soil at PWP (53.2%) followed by PS (23.7%). Loamy sand and sand had no reductions in foliar area associated with *F. graminearum* (Fig. 4B).

Greatest reductions in root dry weight were observed in sandy loam and loam soils at PS and FC, ranging from 2 to 27%. In addition, there was no reduction in root dry weight at PWP across all soil textures (Fig. 4C). Similarly, there were significant reductions in shoot dry weight in sandy loam and loam soils ranging from 3 to 19%. Seedlings growing in loamy sand and sand did not display reductions in shoot dry weight (Fig. 4D).

Greatest reductions in root volume were observed in plants growing in sandy loam soils in all soil moisture treatments, followed by loamy and sandy loam soils at FC and PS (Fig. 4E). Reductions in number of root tips were significantly higher (6.5 to 16.2%) in plants growing in sandy loam soils (Fig. 4F). No reductions in shoot and root morphological characteristics were observed in the sand treatment.

TABLE 4. F values for comparisons of seedling growth parameters between *Fusarium graminearum*-infested and noninfested treatments on the main effects of soil texture and soil moisture (PS = pot saturation, FC = field capacity, PWP = permanent wilting point) at different soil pH^a

		pH 6							
Soil texture	θ_v	RL	FA	RDW	SDW	RV	RT	SA	F
Sand	PS	1.71	0.04	0.83	0.85	0.05	1.18	0.62	2.65
	FC	2.24	2.65	2.48	3.06	0.37	0.95	1.08	3.05
	PWP	2.06	0.50	0.85	0.32	1.94	1.77	2.05	5.06*
Loamy sand	PS	3.27	0.01	3.30	0.41	2.86	0.14	3.56	0.10
	FC	1.76	0.49	0.83	0.17	3.88	0.07	2.95	0.60
	PWP	7.06*	0.66	2.61	0.06	15.75*	0.01	11.37*	0.31
Sandy loam	PS	33.75*	22.67*	26.38*	23.25*	50.74*	9.40*	45.05*	18.17*
	FC	14.98*	1.66	2.06	5.90*	12.76*	7.38*	14.37*	13.33*
	PWP	9.27*	15.20*	3.53	3.92	22.67*	0.66	16.01*	2.85
Loam	PS	10.45*	8.95*	13.51*	5.93*	15.97*	3.25*	13.98*	1.63
	FC	5.38*	0.36	5.08*	0.65	12.98*	1.24	9.13*	2.19
	PWP	2.32	6.52*	0.71	1.78	4.77	0.38	3.84	0.01
		pH 8							
Soil texture	θ_v	RL	FA	RDW	SDW	RV	RT	SA	F
Sand	PS	1.25	1.96	0.17	6.13*	0.04	1.29	0.41	1.03
	FC	0.51	3.81	0.78	11.74*	0.38	0.10	0.49	0.28
	PWP	0.04	8.87*	1.81	2.94	0.05	0.67	0.25	0.70
Loamy sand	PS	1.33	0.36	0.36	0.02	5.48*	1.07	3.26	0.08
	FC	9.90	0.74	0.52	0.13	5.06*	1.13	7.65*	7.29*
	PWP	0.37	0.18	0.04	0.10	1.47	0.84	0.90	0.13
Sandy loam	PS	2.74	3.19	4.68*	2.34	3.89	1.65	3.44	1.39
	FC	8.86*	7.86*	10.93*	13.06*	17.07*	2.46	13.91*	11.32*
	PWP	7.01*	4.48*	0.01	2.04	16.66*	2.84	12.30*	2.84
Loam	PS	0.27	0.01	0.71	0.02	1.45	2.14	0.17	5.15*
	FC	8.78*	3.16	4.93*	0.12	8.60*	5.29*	8.81*	5.46*
	PWP	0.54	0.29	0.39	1.56	2.49	5.71*	0.15	2.40

^a * Indicates significant differences from the noninfested control ($P \leq 0.05$) with a *t* test for mean separation. RL = root length (cm), FA = foliar area (cm²), RDW = root dry weight (g), SDW = shoot dry weight (g), RV = root volume (cm³), RT = root tips, SA = surface area, and F = forks. Data represent values for two experiments with 16 observations per treatment combination. Analyses were performed on square root transformed data.

Comparison between qPCR and visual evaluation of root rot severity. The qPCR protocol was highly sensitive and *F. graminearum* DNA was detected in all the roots grown in artificially infested soils. Plants growing at PWP had the greatest amount of fungal DNA in all the soil textures. However, the highest levels of fungal DNA were observed in sandy loam and loam soil textures at PWP; conversely, a significantly lower amount of fungal DNA was measured in all soil textures at PS and FC (Fig. 5A).

The effects of soil moisture and soil texture on the amount of *F. graminearum* DNA displayed similar trends to the visual evaluation of root rot severity (Figs. 2A and 5A), although fungal DNA was relatively low in sand compared with the root rot ratings. The amount of *F. graminearum* DNA was lowest in sand and generally increased with finer soil textures. Estimations of the amount of *F. graminearum* DNA in the root tissues by the qPCR assay and the visual evaluations of root rot severity in plants

growing on *F. graminearum*-infested soil were positively and significantly correlated ($r = 0.6946$, $P = 0.0121$) (Fig. 5B).

The quantity of fungal DNA in root tissues was negatively correlated with seedling growth parameters including, foliar area ($r = -0.6536$, $P = 0.0211$), root volume ($r = -0.7536$, $P = 0.0046$), shoot dry weight ($r = -0.7117$, $P = 0.0091$), root length ($r = -0.8092$, $P = 0.0014$), and root dry weight ($r = -0.8791$, $P = 0.0001$) (Fig. 5C and D).

DISCUSSION

In this study, we provided the first analysis concerning the interactions among several abiotic soil factors (soil moisture, soil pH, and soil texture) in the development of root rot of soybean seedlings caused by *F. graminearum*. The results suggest that edaphic characteristics have a considerable influence on disease

Fig. 1. Effects of soil pH on root rot of soybean caused by *Fusarium graminearum*. Average values of visual disease evaluations over **A**, soil moisture content and **B**, soil texture. Means with the same letter are not significantly different according to Fisher's protected least significant difference ($P < 0.05$). Comparisons were made separately by pH level on the arcsine transformed data. Data represent values of two experimental replications. Analysis did not include data from noninfested plants. PS = pot saturation, FC = field capacity, and PWP = permanent wilting point.

Fig. 2. Combined effects of soil texture and soil moisture content on root rot of soybean caused by *Fusarium graminearum*. Average values of visual disease evaluations on **A**, soil at pH 6 and **B**, soil at pH 8. Means with the same letter are not significantly different according to Fisher's protected least significant difference ($P < 0.05$). Comparisons were made separately by pH level on the arcsine transformed data. Data represent values for two experimental replications and do not include data from noninfested plants. PS = pot saturation, FC = field capacity, and PWP = permanent wilting point.

severity and seedling development. A somewhat acidic soil pH (pH 6) was more conducive for root rot than an alkaline pH (pH 8). Root rot severity was significantly affected by the soil moisture treatments, of which PWP displayed the highest levels of root rot and PS the lowest, suggesting that moisture-stress conditions favor development of root rot caused by *F. graminearum*.

Our findings that disease was more severe under acidic soil conditions (pH 6) agree with several similar studies. For example,

sorghum root infection by *F. graminearum* was significantly higher at soil pH 5.1 than at pH 6.1 on seedlings 36 days after planting under field conditions (Davis et al. 1994). Strawberries infected by *F. oxysporum* f. sp. *fragariae* displayed more severe root rot and reduced plant size at pH 5.2 than at 7.5 (Fang et al. 2012). Increasing soil pH has been suggested as an alternative for disease management in other Fusarium wilt diseases such as banana, spinach, tomato, cotton, and melons (Dominguez et al. 1996; Gatch

Fig. 3. Root and shoot growth measurements in *Fusarium graminearum*-infested and noninfested treatments according to the main effects of soil texture and soil moisture on **A**, root length (cm), **B**, foliar area (cm²), **C**, root dry weight (g), **D**, shoot dry weight (g), **E**, root volume (cm³), and **F**, root tips. * Indicates significant differences from the noninfested control ($P = 0.05$) with a t test for mean separation. Mean comparisons were performed individually by soil moisture on each soil texture. Analyses were performed on the square root transformed data. Data represent values for two experimental repetitions, each with four replicates, including data from soil pH 6 and 8. Noninfested control contained sterile noninfested millet (10%, vol/vol). PS = pot saturation, FC = field capacity, and PWP = permanent wilting point.

and du Toit 2017; Groenewald et al. 2006; Jones et al. 1989; Woltz and Jones 1973). It seems plausible that increasing soil pH with CaCO_3 might have reduced growth of *F. graminearum*, decreasing the chances to effectively infect soybean roots.

Previous reports suggest that increasing soil pH by liming reduces soil fungal populations and at the same time improves

bacterial growth (Alabouvette 1999; Muhlbachova and Tlustos 2006; Scher and Baker 1980). According to our observations, increasing the soil pH with CaCO_3 significantly reduced root rot severity caused by *F. graminearum* during a 4-week period.

High soil pH can lead to IDC in soybean, which is associated with root rot symptoms often attributed to *Fusarium* spp. However, our

Fig. 4. Root and shoot growth reductions (%) in *Fusarium graminearum*-infested treatments relative to the noninfested control according to the main effects of soil texture and soil moisture on **A**, root length, **B**, foliar area, **C**, root dry weight, **D**, shoot dry weight, **E**, root volume, and **F**, root tips. Means with the same letter are not significantly different according to Fisher's protected least significant difference ($P < 0.05$). Analyses were performed on the square root transformed data. Data represent values for two experimental replications including data from soil pH 6 and 8. Noninfested control = soil containing sterile noninoculated millet (10%, vol/vol). PS = pot saturation, FC = field capacity, and PWP = permanent wilting point.

results do not support a role for *F. graminearum* in enhanced root rot symptoms under high pH conditions. Root rots associated with IDC are likely caused by other fungal species (Hansen et al. 2004).

In our studies, the effect of soil moisture differed among the soil types. In sand, root rot severity was relatively high for all soil moisture levels; the PWP treatment had the highest value but was not significantly different from FC or PS. In loamy sand, PWP had the highest severity and there was no difference between FC and PS at pH 6. In the finer-textured soils (sandy loam and loam at pH 6; loamy sand, sandy loam, and loam at pH 8), there was a distinct pattern of increasing root rot with decreasing soil moisture. Together, these results reinforce the conclusion that root rot was enhanced under moisture-stress conditions. In sand, plant moisture stress may have been similar in all the moisture content treatments, due to rapid drainage of water added to the cups. The interaction of soil texture with soil pH was not significant, but a trend toward lower root rot severity with higher clay content was more evident at pH 8 than at pH 6. This observation is consistent with previous reports by Hoper et al. (1995) and Amir and Alabouvette (1993) on *Fusarium* wilts, which demonstrated that addition of clay (montmorillonite or illite) provides the most disease suppression at high soil pH.

Soil texture affects plant disease due to the correlation with water holding capacity, nutrient availability, porosity and root growth

(Ghorbani et al. 2008). In our studies, we observed that the combined effect of soil texture and soil moisture displayed a marked influence on disease severity. For example, there were no significant differences in root rot across soil moisture contents in sand and this may be partially attributed to the fact that the volumetric water content differed very little among soil moisture treatments ranging from 0.07 to 0.13. In addition, the highest levels of root rot were observed in soil treatments with volumetric water contents equal to or below 0.13. Conversely, the effects of soil moisture treatment on root rot severity became more accentuated with incrementally higher clay content across soil textures, suggesting that the water holding capacity effect of clay played an important role in avoiding moisture stress conditions that favored root rot by *F. graminearum*.

Root rot was significantly higher at PWP soil moisture treatments in all soil textures except sand. Similar results regarding drought stress predisposing soybean to root infection by diverse *Fusarium* spp. have been described by Han et al. (2017). Similarly, in cereals, *F. graminearum* tends to be the most predominant *Fusarium* species in the warmer regions of the United States, and warm and dry weather is considered to be the optimal condition for root rot development (Beddis and Burgess 1992; Doohan et al. 2003; Vigier et al. 1997). It is likely that there is an association between dry climatic conditions optimal for *F. graminearum* to produce disease and our observation on root rot of soybean.

Fig. 5. Relationships between fungal DNA quantification in root tissue, edaphic factors and seedling growth parameters from plants growing in *Fusarium graminearum*-infested soil at pH 6. **A**, Combined effects of soil texture and soil moisture content on the amount of fungal DNA. **B**, Relationship between visual evaluations of root rot severity (%) and the amount of fungal DNA. **C**, Relationship between root length and amount of fungal DNA. **D**, Relationship between root weight and amount of fungal DNA. Each mean and point represent the average of 24 plants collected 30 days after planting. Means with the same letter are not significantly different according to Fisher's protected least significant difference ($P < 0.05$). PS = pot saturation, FC = field capacity, and PWP = permanent wilting point.

Soybean seedlings growing in soils at PWP displayed reduced root and shoot growth. The effects of low water stress in soybean include inhibition of cell division, reduction in foliar area, suppression of shoot, root growth, and root volume, and decrease in photosynthetic rate (Hossain et al. 2014; Krizek et al. 1985). Although studying the adverse effects of water stress on soybean seedling growth parameters was not our main objective, it is important to note that the highest levels of root rot were observed on water-stressed seedlings with reduced growth in root and shoot. For these reasons, it was more informative to draw conclusions on the effects of our treatments on plant growth variables by making comparisons between the infested and noninfested seedlings in each treatment combination. In this way we could evaluate the effects of the treatments on the disease, rather than their direct effects on seedling growth.

Soybean seedlings growing in *F. graminearum*-infested soils displayed significant reduction in plant growth parameters. These findings are in general agreement with Zhang et al. (2010) and Xue et al. (2007), in which seed or seedling *F. graminearum* inoculation reduced root dry weight and plant height up to 24 and 28%, respectively. Reductions in root length, root volume, number of tips and forks by *F. graminearum* soil infestation has also been reported (Díaz Arias et al. 2013a). Differences in all plant growth parameters between infested and noninfested plants in sand were negligible. This effect may be due in part to the low impedance for root elongation offered by the high sand content allowing the roots to scavenge, reducing detrimental effects of *F. graminearum*, also evident in the shoot weight and foliar area (Ballgar et al. 1980).

Highly significant differences were observed between the infested and noninfested treatments for all the root morphological variables in the PS and FC sandy loam soils, even though these treatment combinations had the lowest root rot severity. A possible explanation for the correlation between low root rot severity and high percentage of growth reduction is that the visual evaluation of disease does not take into account the loss of fine roots (or their failure to develop) when root infection takes place. It also does not account for internal root colonization and the possible damage in the vascular system that might affect root growth and development.

Root morphological characteristics analyzed on WinRhizo are useful indicators of root health. Estimates of root length, and root volume, root forks and root tips helped to interpret the detrimental effects of *F. graminearum* and its interactions with soil moisture, soil texture, and soil pH. Root volume, however, seems to be the most sensitive variable in estimating detrimental effects of root rot. For example, there were significant differences in root volume comparing infested and noninfested treatments in all soil textures and soil moisture treatment combinations except for sand.

The amount of *F. graminearum* DNA was highly affected by the edaphic factors and demonstrated similar trends as the visual observations of root rot severity. The positive Pearson's correlation coefficients obtained from the comparison between the fungal DNA quantities and the visual observations of root rot severity evidence the qPCR assay's ability to quantify the target DNA and to indicate the extent of *F. graminearum* root colonization 30 days after planting. These results are consistent with those of Zhu et al. (2016), who reported significantly positive correlations between disease severity ratings and DNA quantities of *F. commune* in stem tissue of Chinese water chestnut (*Eleocharis ducis*).

Correlations between seedling growth parameters and fungal DNA quantities were significantly negative. These correlations correspond to short damaged roots with a high degree of fungal colonization and long, healthier roots harboring low quantities of fungal DNA. The best correlations between DNA quantity and root rot or root growth parameters occurred in the PWP treatments, which also displayed the highest of values for root rot and root growth. These results suggest that water-stressed seedlings are more predisposed to *F. graminearum* colonization, and the extended *F. graminearum* root colonization results in smaller underdeveloped

roots. Similar observations have been described in which *Fusarium* spp. were more frequently isolated and able to colonize to a greater degree roots of well-drained soils resulted in lower root growth (Ali and Ghaffar 1991; Beddis and Burgess 1992; Han et al. 2017).

Additional studies will be needed to determine if the correlation between DNA quantities and visual observations of disease remain significant in later soybean stages. There is evidence that suggests that *Fusarium* spp. endophytic root colonization is dynamic and weather-dependent in other pathosystems (Bateman and Murray 2001; Hogg et al. 2010). It will be necessary to understand the impact of water stress in the root and rhizosphere under water stress that make the plant more susceptible to *F. graminearum* infection. Nevertheless, the evidence from our studies suggests that the ability of *F. graminearum* to colonize root tissues depends on the combination of edaphic factors, and these findings might have direct implications for future studies of *F. graminearum* populations.

The results from this study have provided information on abiotic factors of importance for root rot of soybeans caused by *F. graminearum*. It was demonstrated that changes in edaphic factors and their interactions influence disease severity and fungal root colonization. The soil parameters studied the most in relation to soilborne pathogens are soil pH, clay content, and nitrogen content (Janvier et al. 2007). There are not many reports on *Fusarium* root diseases that included several soil factors in the same study and a combined approach is needed due to the possible interactions among biotic and abiotic factors related to soilborne diseases.

Additional variables such as temperature fluctuation and interactions between and within fungal species are needed. For example, Tu (1994) studied the effects of soil moisture and temperature on the *Fusarium* root rot complex of pea, including *F. solani* f. sp. *pisi* and *F. oxysporum* f. sp. *pisi* and found that both lack of moisture and saturation increased disease severity. It is important to study these types of interactions in the future since the soybean root rot complex of soybeans is not only associated with *F. graminearum* but includes a diverse number of *Fusarium* species (Díaz Arias et al. 2013a; Leslie et al. 1990).

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