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Influence of Soil Moisture on Root Colonization of Glyphosate-Treated Soybean by *Fusarium* Species

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Abstract: The widespread use of glyphosate-resistant (GR) cropping systems may impact rhizosphere microbial associations and crop productivity. It was previously reported that glyphosate accumulation in the rhizosphere may stimulate colonization of soybean [*Glycine max* (L.) Merr.] roots by soilborne *Fusarium*. Field studies often reveal inconsistent root colonization by *Fusarium*, especially during growing seasons characterized by contrasting rainfall patterns. Therefore, this study was conducted to determine the impact of different soil moisture contents on root colonization of glyphosate-treated soybean by *Fusarium* species. Glyphosate (0.84 kg ae ha^{-1}) was applied to greenhouse-grown glyphosate-resistant (GR) soybean at the two to three trifoliate-leaf (V2–V3) growth stage growing in a Mexico silt loam at 27%, 13%, and 10% soil moisture contents. Soil and plant samples were sampled periodically after herbicide application and selectively cultured for *Fusarium*. Highest *Fusarium* colonization was associated with the glyphosate treatment, with maximum levels occurring at the highest soil moisture level. Thus, glyphosate interactions with root colonization by *Fusarium* in glyphosate-resistant soybean are greatly influenced by soil moisture content.

Keywords: *Fusarium*, glyphosate, glyphosate-resistant soybean, soil moisture, rhizosphere

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INTRODUCTION

Glyphosate [*N*-(phosphonomethyl)glycine, Roundup®], a broad spectrum, nonselective herbicide for postemergent control of a wide range of weeds, is the most widely used herbicide because of the introduction and broad acceptance of genetically modified (GM) glyphosate-resistant (GR) crop varieties in the late 1990s. On a global basis, soybean is the most prevalent GM crop in GR cropping systems, planted on 60% of the global GM-cropped land in 2005 (Crop Biotech 2006). Glyphosate is systemic and not readily metabolized by plants; it is translocated and may accumulate in meristematic regions including roots and nodules (Duke 1988; Hernandez, Garcia-Plazaola, and Becerril 1999; Reddy and Zablotowicz 2003). Glyphosate that accumulates in the roots of susceptible plants is eventually released into the rhizosphere (Coupland and Casely 1979; Rodrigues, Worsham, and Corbin 1982). Field and laboratory studies have shown that glyphosate directly increases soil bacterial and fungal populations, possibly serving as a nutrient source for microbial growth (Wardle and Parkinson 1992a; Haney et al. 2000; Busse et al. 2001).

Previous research demonstrated that glyphosate applied to soil (Abdel-Mallek, Adbel-Kader, and Shonkeir 1994) and susceptible plants (Lévesque, Rahe, and Eaves 1987; Kawate et al. 1997) increased soil and rhizosphere *Fusarium* populations within a week after application. Glyphosate added to sandy clay from crop fields previously treated with glyphosate exhibited a selective effect for specific fungal species, including *Fusarium* spp., that were able to use the herbicide as a nutrient source (Krzyśko-Lupicka and Orlik 1997). Elevated levels of glyphosate (9.2 mg kg dry soil^{-1}) delayed decomposition of plant residues (Abdel-Mallek, Adbel-Kader, and Shonkeir 1994), suggesting *Fusarium* preferentially utilized glyphosate as a nutrient source before attacking plant residues.

The previous studies established the existence of a glyphosate–*Fusarium* interaction. Regarding GR crops, Kremer (2003) found populations of *Fusarium* increased 0.5- to 5-fold on GR soybean roots 2 or 4 weeks after glyphosate application. The release of glyphosate into GR soybean rhizospheres was also documented, suggesting that the composition of root microbial communities could shift to favor those that utilize glyphosate as a nutrient source (Kremer, Means, and Kim 2005). Furthermore, glyphosate may enhance the growth of certain microorganisms, including *Fusarium* spp., that may be pathogenic to GR soybean and cause a buildup of detrimental species that affect subsequent crops.

The first growing season of widespread cultivation of GR soybean in 1997 was unusually wet in the midwest United States, which contributed to severe epidemics of soybean sudden death syndrome (SDS) caused by the soilborne fungal pathogen *Fusarium solani* f. sp. *glycines* (Wrather, Stienstra, and Koenning 2001). Glyphosate-resistant varieties were more frequently identified with SDS than conventional varieties (Myers et al. 1999). These observations suggested that *Fusarium* infection was related to soil moisture

content (SMC) and to the increased use of glyphosate with GR soybean. Our recent field studies revealed inconsistent colonization of roots by *Fusarium* between growing seasons, partly due to contrasts in seasonal rainfall patterns (Means 2004).

Studies on soil moisture relationships with root colonization of GR soybean by *Fusarium* species are very limited. Thus, our research objective was to determine the impact of different SMCs on root colonization of glyphosate-treated soybean by *Fusarium* species.

MATERIALS AND METHODS

Soil collected from the A horizon of a Mexico silt loam (fine, smectitic, mesic Aeric Vertic Epiaqualfs) was placed in plastic containers (8000 cm^3; 20 cm in diameter and 30 cm in height) and packed to establish a bulk density of 1.3 g cm^{-3}. Selected soil properties were 7.1 pH_s, 3.3% organic matter, 75 kg ha^{-1} phosphorus (P) (Bray 1), 4240 kg ha^{-1} calcium (Ca), 500 kg ha^{-1} magnesium (Mg), 300 kg ha^{-1} potassium (K), and 13.1 cmol kg^{-1} cation exchange capacity (CEC). A 200-mL soil suspension (250 g of fresh surface soil collected from plots in a corn and soybean rotation on a Mexico silt loam mixed with 5 L of tap water) was added to each container to ensure the presence of representative microbial populations under field conditions. Five to seven GR soybean (DeKalb DKB38-52) seeds were planted 2.5 cm deep in each container. All containers were maintained under greenhouse conditions where day and night temperatures averaged 30 and 15°C, respectively. Soil moisture contents were maintained during the experiment by monitoring each container daily with analog soil moisture testers (SMTs) (Spectrum, Plainfield, Ill.) (The mention of trade or manufacturer names is made for informational purposes only and does not imply an endorsement or exclusion by the USDA-ARS or the University of Missouri.) Prior to beginning the experiment, SMTs were calibrated to desired SMCs by repeated sampling of the prepared Mexico silt loam and determining gravimetric soil moisture. Soil moistures were selected to represent "well watered" (27% gravimetric soil moisture), "some water deficit" (13%), and "extreme water deficit" (10%) conditions. Glyphosate (Roundup Ultra-Max®) was applied based on label rate of 0.84 kg ae ha^{-1} using a pressurized sprayer when plants were at the V2–V3 growth stage (Hanway and Thompson 1971). Plants in control treatments did not receive glyphosate.

Single intact plants were collected from each container at 0, 10, 20, and 30 days after glyphosate application. Loosely adhering soil on roots was removed by vigorous shaking. Aboveground portions of soybean plants were severed at the soil line, placed in paper bags, and dried for 24 h at 50°C to determine plant biomass dry weight. Soybean roots were surface sterilized in 1.25% sodium hypochlorite for 2 min followed by rinsing three times in sterile water. Roots were blotted dry on sterile paper towels and cut into 2-cm segments; eight

segments were placed onto a single agar plate of Komada medium, which is selective for *Fusarium* spp., (Mekwatanakarn and Sivasithamparam 1987). Two plates were prepared for each plant and incubated for 5 days at 25–30°C with 12 h of indirect sunlight (Mekwatanakarn and Sivasithamparam 1987). A *Fusarium* colony that developed on a root segment was counted as a single colony-forming unit (cfu) of rhizoplane *Fusarium*. Total numbers of *Fusarium* colonies per plate or 16 cm of root were converted to *Fusarium* cfu per 100 cm of root. *Fusarium* colonies were randomly selected, subcultured on potato dextrose agar, and tentatively identified using descriptions of cultural and microscopic morphologies (Nelson, Toussoun, and Marasas 1983). Identification of isolates was confirmed at the USDA-ARS Microbial Genomics Unit, Peoria, Ill., by molecular analysis using partial translation elongation factor sequences (Skovgaard et al. 2001).

The experimental design was a repeated measures (four sample dates) split-plot arrangement with two treatments (glyphosate and no herbicide) and three soil water contents (27%, 13%, and 10% soil moisture) for a total of six treatments with four replications each. The greenhouse experiment was conducted twice during May–July of both 2004 and 2005. Data presented in the figure are mean values of four independent greenhouse replicates. A general linear model was used to analyze the data. Analysis of variance and mean separations (Fisher's protected least significant differences; LSD) were made using SAS (SAS Institute, Cary, N.C.). It was found that the data were normally distributed with similar variances.

RESULTS AND DISCUSSION

Fusarium colonization of soybean roots was similar at all SMCs at the two initial sampling dates regardless of glyphosate application (Figure 1). Beginning at 20 days post–glyphosate application, *Fusarium* colonies increased twofold on roots of soybean plants treated with glyphosate at all three soil water contents compared with untreated plants. Total colonization of soybean root varied with SMC, especially under glyphosate treatment. For glyphosate-treated plants, *Fusarium* colonization ranged from about 80–150 colonies per 100 cm of root at 13 or 27% soil moisture to 55–65 colonies per 100 cm of root at 10% soil moisture (Figure 1). In contrast, *Fusarium* colonization of plants without glyphosate treatment was consistent among all soil moisture contents at $\leq$50 colonies per 100 cm of root throughout the sampling period. The wide difference in *Fusarium* colonization between glyphosate-treated and untreated plants confirms previous field observations (Kremer 2003; Means 2004); however, depression of colonization under moisture stress (10% soil moisture) is documented for the first time. Based on morphological and molecular analyses, 80% of the isolates were *Fusarium oxysporum*; *F. solani* and *F. equiseti* each comprised 10% of the isolates.

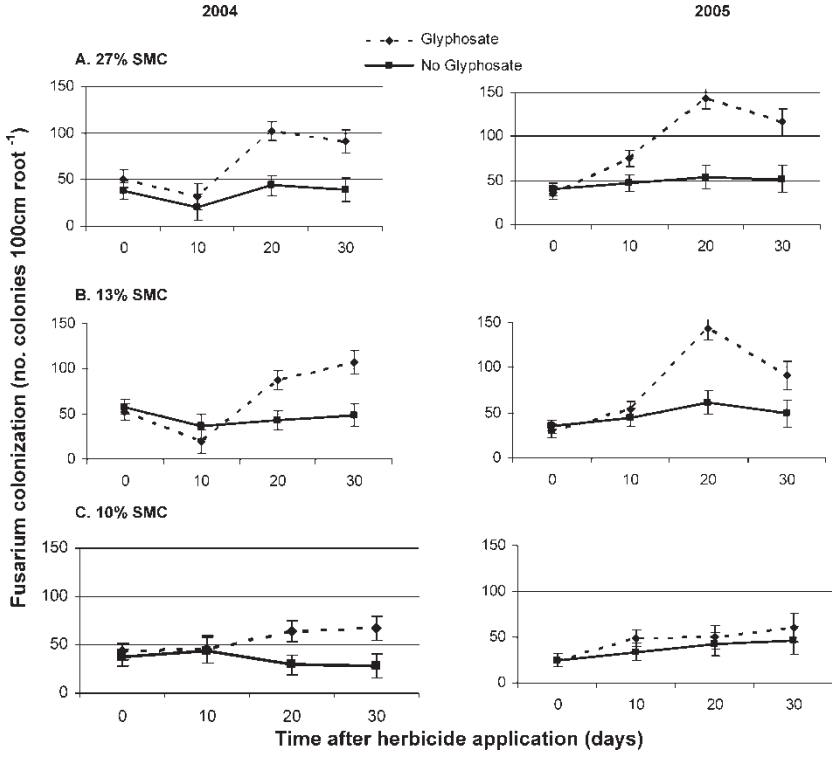

Figure 1. Relationship of glyphosate application and three soil moisture contents (SMCs) with colonization of glyphosate-resistant soybean roots by *Fusarium*. Vertical bars indicate significant differences ($P < 0.05$) between treatments within a soil moisture level based on Fisher's protected LSD.

Fusarium colonization of soybean roots was not correlated with aboveground biomass accumulation. Aboveground biomass decreased with decreasing levels of soil moisture regardless of glyphosate treatment (data not shown). Aboveground biomass was similar for plants grown within the same SMC; plants grown at 27% soil moisture accumulated approximately three times the biomass as those grown at 13 and 10% soil moisture.

Our results agree with previous field research that reported increased rhizosphere colonization by *Fusarium* (a) of soybean with increasing SMCs (Sanogo, Yang, and Scherm 2000; Kremer 2003) and (b) of GR soybean following glyphosate application (Cheng and Schneck 1978; Scherm and Yang 1996; Scherm, Yang, and Lundeen 1998). Glyphosate applied to plants appeared to influence *Fusarium* through several mechanisms. Glyphosate may increase the quantity and alter the composition of substances released by root exudation and ultimately enhance *Fusarium* growth in the rhizosphere (Greaves and Sargent 1986; Kremer, Means, and Kim 2005). Host plant

defenses may be weakened from reduced phytoalexin production, caused by interruption of the shikimate pathway by glyphosate, and allow increased root colonization by *Fusarium* (Johal and Rahe 1988). Also, glyphosate may be translocated to roots, released into the rhizosphere, and selectively stimulate *Fusarium* growth and root colonization (Wardle and Parkinson 1992b; Krzyśko-Lupicka and Orlik 1997; Kremer, Means, and Kim 2005).

The combined effects of glyphosate with optimum to high soil moisture that led to increased *Fusarium* populations (root colonization) in this study may help explain the *Fusarium* disease epidemics documented in GR soybean fields during wet growing seasons, notably in 1997, the first year of widespread GR soybean production (Wrather, Stienstra, and Koenning 2001). High root colonization rates by *Fusarium* species under optimum soil moisture also leads to reduced root biomass (Ortiz-Ribbing and Eastburn 2004). Thus, decreases in root mass by glyphosate may be augmented by coincident enhancement of *Fusarium* colonization, leading to reduced plant productivity. At low soil moisture, however, soybean root biomass was likely decreased before or during *Fusarium* colonization, which resulted in the decreased colonization observed at these moisture contents.

In summary, increased *Fusarium* colonization was associated with glyphosate treatment with maximum populations at optimum to high SMCs. The information obtained in this study on the influence of soil moisture on glyphosate interactions with *Fusarium* colonization of GR soybean roots is useful in partly explaining apparent root disease problems often observed in GR soybean on wet soils. The results further suggest that management practices should be developed to carefully consider soil moisture characteristics of specific sites within fields under GR soybean production.

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