Discovery, The Student Journal of Dale Bumpers College of Agricultural, Food and Life Sciences

Volume 8 Article 9

Fall 2007

Mycorrhizal infection rates in RoundupReady® row crops in response to glyphosate and phosphorus applications

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Mycorrhizal infection rates in RoundupReady® row crops in response to glyphosate and phosphorus applications

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ABSTRACT

Currently, the majority of soybean, corn, and cotton crops grown in the U.S. is RoundupReady® (RR) varieties. RR crops are resistant to the active ingredient, glyphosate [N-phosphonomethyl-glycine], in the herbicide Roundup®. RR crops have been genetically modified by the addition of an enzyme found in *Agrobacterium* sp. strain *CP4 EPSPS* that produces an essential protein, involved with aromatic amino-acid production, that is resistant to glyphosate. Glyphosate translocates via phloem from plant leaf tissues to other areas including the root system, and is thus able to affect the rhizosphere microbial community, including mycorrhizae, which are not resistant to glyphosate. A greenhouse experiment was conducted to determine response of mycorrhizal infection and plant nutrients to glyphosate and phosphorus (P) applications to RR soybean, corn, and cotton. Crops were untreated, or treated with glyphosate in low-P soil or in P-fertilized soil, and grown for 6 weeks, after which roots and shoots were harvested and analyzed for mycorrhizal infection and P concentrations. Plant roots were cleared and stained with Trypan Blue dye and analyzed with a dissecting microscope for mycorrhizae on a percent-root basis. Phosphorus had significant positive effects on plant shoot P concentrations for all crops. Mycorrhizal infection rates showed a negative effect in soybean with reduced infection in the P treatment. Glyphosate for all crops and all treatments showed no effect on mycorrhizal infections or plant shoot-P concentrations. Therefore, our results indicate that glyphosate generally may be disregarded in terms of potential detrimental effects on mycorrhizal-plant interactions or plant-P uptake by soybean, corn, and cotton crops in low-P soil.

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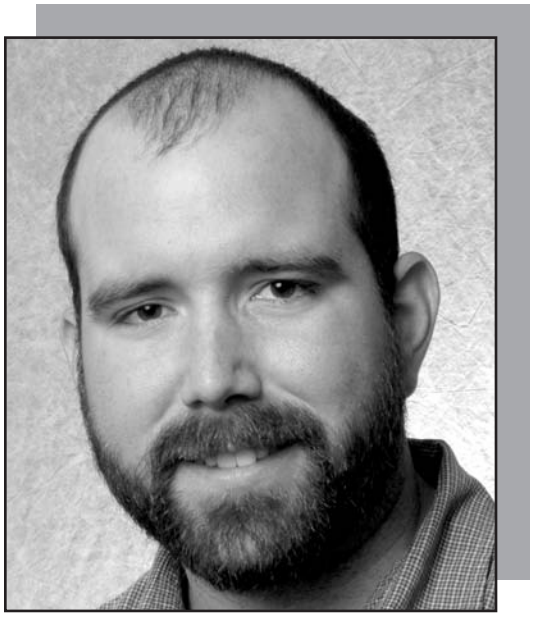
INTRODUCTION

In recent years, since introduction in 1996, the use of RoundupReady® (RR) varieties has increased in popularity and RR cultivars yield the majority of soybean (*Glycine max*), corn (*Zea mays*), and cotton (*Glossypium hirsutum*) crops. In 2002, 72% of U.S. crop hectares consisted of RR crops (USDA-AMS, 2002). RoundupReady® crops are resistant to the active ingredient glyphosate [N-phosphonomethyl-glycine] in the herbicide Roundup® (Monsanto Co., St. Louis, Mo.). Roundup® has gained favor with farmers because of the susceptibility of a broad range of weeds to it and because, being a post-emergence foliar herbicide, there is an increased window of time for application; all of which reduce maintenance, labor, costs, and mixing of numerous herbicides to target multiple weed species, and supports conservative tillage practices (Moschini et al., 2000). Roundup® is also less environmentally persistent because it is degraded relatively quickly once in contact with soil microbes.

Glyphosate targets and inhibits a specific protein and protein derivatives involved in biosynthesis of aromatic amino acids in all plants (Heck et al., 2005). RoundupReady® crops have been genetically modified by the addition of an enzyme found in a strain of *Agrobacterium* sp. *CP4 EPSPS* that produces a glyphosate-resistant transgenic protein, 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) (Barry et al., 1992; Padgette et al., 1995). Therefore, expression of the resistant EPSPS maintains the aromatic amino-acid biosynthetic pathway, which prevents plant death and preserves crop quality, nutritional health, and yields (Delannay et al., 1995; Hammond et al., 1995; Harrison et al., 1996; Padgette et al., 1995; Nida et al., 1996).

Glyphosate translocates via phloem from plant leaf tissue to other areas including the root system (Hetherington et al., 1999). As a non-selective herbicide, glyphosate is suspected to be detrimental to other living organisms, including microbial communities. The plant-soil interface encompasses increased levels of diverse microbial activity, including mycorrhizae.

MEET THE STUDENT-AUTHOR



Aaron L. Daigh

I am from Fayetteville, Ark., and graduated from Farmington High School in 2003. I enrolled as a chemical engineer major in fall 2003 and became an environmental, soil, and water sciences major in fall 2005. In May 2007 I will graduate with a B.S.A. in environmental, soil, and water sciences and a minor in wildlife habitat management. I served as the Crop, Soil, and Environmental Sciences (CSES) Club president in 2006 and helped the club obtain the Fayetteville Parks and Recreation Volunteer Group of the Year award along with 1st and 2nd place awards for the respective 2005 and 2006 American Society of Agronomy National Club Poster Symposium. I received the Delta Classic Scholarship from CSES, White River Environmental Protection Association Scholarship, James L. Gray Scholarship from the Arkansas Association of Professional Soil Classifiers, and the CSES Outstanding Senior Award.

From fall 2005 until the present, I have worked for Dr. Mary Savin in soil microbiology and ecology. I also worked for Dr. Chuck West in forage science in spring 2006 and Dr. Larry Purcell as part of this project. While working for Dr. Savin and Dr. Purcell, I began research with mycorrhizal responses to glyphosate and phosphorus, and continued into a new research project investigating urea-N mobility in rice soils. I presented research results in the American Society of Agronomy Undergraduate Research Symposium Contest and received a Dale Bumpers College of Agricultural, Food, and Life Sciences Undergraduate Research Grant in fall 2006. I plan to further my education in graduate school with research focused within the soil sciences.

Mycorrhizae are symbiotic fungi involved in intimate exchange of nutrients directly with plant root systems. Mycorrhizae live both within and outside the structure of plant roots. Hyphae of the mycorrhizae extend outward from roots into the soil, functioning similarly to root hairs, as extensions of the root system by increasing surface area and the uptake of water (H_2O) and essential nutrients (particularly phosphorus, P). Studies have shown that adequate P uptake in most of the world's plants is dependent on mycorrhizae-root interaction (Janos, 1980; Hartnett et al., 1993; Koide et al., 1994). Mycorrhizae exchange H_2O and P for carbon in the form of carbohydrates created by photosynthesis within a plant. This intimate relationship between mycorrhizae and the plant generates concern for susceptibility of the fungus to damage attributed to glyphosate application because of the possible translocation of compounds from leaves to the root system.

Currently, over 90% of all soybean crops and increasing numbers of corn and cotton crops within the United States use RR varieties. Due to increased use nationwide of glyphosate-resistant crop varieties, any changes within the rhizosphere communities, particularly to mycorrhizae, could have potential economic consequences. In the event of a detrimental response by mycorrhizal symbionts to glyphosate products, decreased levels of P fertilizer uptake by plant roots are probable and would contribute to P nutrient deficiencies within crops. In the event of P deficiencies following glyphosate applications, farmers would need to apply additional P to fields to maximize plant biomass and crop yield. In the event of no significant detrimental effects to mycorrhizal symbionts after glyphosate applications, then use of conservative P application rates can be considered.

We hypothesized that mycorrhizal sensitivity to glyphosate would decrease mycorrhizal infection in RR crops sprayed with glyphosate. Predictions were that 1) mycorrhizal infection rates will decrease following application of glyphosate to RR row crops, 2) P uptake will decrease from suppression of mycorrhizal infection, and 3) negative effects of glyphosate on mycorrhizae and plant-P nutrition will be overcome by P fertilization.

MATERIALS AND METHODS

The experimental design consisted of a randomized complete block with four treatments in a low-P soil (11 mg P/kg) obtained from a rice field (Hilleman silt loam) in Poinsett County, Ark. Treatments consisted of 1) no added P (0P) and no glyphosate (0Gly) (control); 2) added P as KH_2PO_4 (1P) and 0Gly; 3) 0P with glyphosate (2Gly); and 4) 1P with 2Gly (Table 1). P was added at a rate of 45 kg P/ha, and glyphosate was applied at about 10 and 20 d after emergence at a rate of 1.1 kg/ha. All treatments were used on three plant species: RoundupReady (RR) A4801 soybean, Garst 8553RR corn, and Paymaster 1218RR/GB cotton.

Soil was sieved and placed in 25-cm-diameter pots. A saucer was placed under each pot to retain water and nutrient solutions. Nitrogen (N) fertilizer (as urea) was applied at 112 kg N/ha to corn and cotton pots by placing solution into the saucer and allowing the solution to be taken up by the plants through the bottom of the pots. A symbiotic bacterium, *Bradyrhizobium japonicum* (strain USDA 110) culture, was added to soybean at time of planting seeds to promote optimal nodulation for N_2 fixation. Phosphorus was applied to appropriate treatments in a similar manner as N. Treatments with no added P received 500 ml DI H_2O to keep inputs consistent and maintain similar soil moisture for all pots. Pots were allowed to sit overnight before seeds were planted. Soybean, corn, and cotton seeds were planted at depths of 1.9 cm, 3.8 cm, and 1.3 cm, respectively. For all crops, 4 seeds were initially planted and were thinned to 1 plant per pot before glyphosate application. All pots were watered 2 times per week when the top 2.5 cm of soil was dry.

Glyphosate was applied 10 and 20 days after unfolding of first true leaves for soybean and corn and after the unfolding of the fourth true leaves for corn. Glyphosate was applied at 1.1 kg/ha as Roundup Original Max® in a solution with the aid of a hand-held boom. All plants were allowed to grow for 6 weeks after emergence before harvesting. At harvesting, plant roots were removed manually from the soil, washed with deionized (DI) H_20, and stored in plastic bags on ice until transported to a refrigerator (4°C). Plant shoots were cut and dried at 65°C before being analyzed for P and N. Plant-shoot P was determined by HNO_3 digestion and analyzed on an inductively coupled plasma spectrophotometer (Spectro Analytical Instruments, Fitchburg, Mass.). Total plant-shoot N was determined by the Dumas method with a Leco FP-428 Determinator (Leco Corporation, St. Joseph, Mo.). Plant-shoot P and N were analyzed by the University of Arkansas Soil Testing and Plant Analysis Laboratory, Fayetteville.

For mycorrhizae analysis, a subsample was taken from each plant root system for clearing and staining (Koske and Gemma, 1989). Subsamples were cleared of pigment with 1.8 *M* KOH in test tubes placed in an 80°C water bath for 15 min. Samples were then washed with DI H_20 and soaked in a 3% bleach solution until transparent. Samples were washed again with DI H_20 and dipped in 5 *M* HCl before being placed into a test tube

containing Trypan Blue dye solution. Samples in the dye were then placed into an 80°C water bath for 30 min. Samples were washed and stored in plastic bags in a refrigerator until further analysis.

Infection rates were measured on a percent-root basis with the method used by Giovannetti and Mosse (1980). A 0.50-g portion of each sample was placed in a petri dish containing a grid with 1.27-cm spacing. The sample was spread evenly within the petri dish. A compound microscope was then used to manually count total, infected root intersects over the grid lines. The method allowed determination of percent mycorrhizal infection. Mycorrhizal structures were determined by the presence of hyphae, vesicles, arbuscules, and/or spores (Fig. 1). Significant differences among treatments for each crop species were determined using PROC GLM in SAS with separation of means using least significant differences ($P < 0.05$).

RESULTS AND DISCUSSION

For all treatments and crops, no significant differences were observed for plant nitrogen concentrations, root weights, and shoot biomass (data not shown). Amended P treatments for all crops showed significant increases in plant-shoot P concentrations compared to treatments of no additional P (Fig. 2). However, glyphosate applications did not have a significant impact on plant-shoot P concentrations on any crop species. Increased plant-shoot P concentrations with fertilizer P were greatest for corn, followed by cotton and then soybean. The highest rates of mycorrhizal infection were observed in cotton, followed by corn and then soybean. Mycorrhizal infection rates showed no significant differences for all treatments for corn and cotton (Fig. 3). However, a significant difference was seen in soybean, with a decrease in mycorrhizal infection rate when P was added. Soybean was, therefore, less reliant on mycorrhizae as an aid to acquire P due to adequate uptake by the root system, and therefore showed a decrease in mycorrhizae interaction.

Significant differences in plant-shoot P between 0P soil and the amended-P soil suggest that soil-P was a limiting factor to plant uptake within the experiment. Plant health and ability to carry out normal and vital functions, such as building of DNA and RNA, increase along with increases in P concentrations unless the plant reaches a state of P toxicity (varies among plant species) (Havlin et al., 2005). Glyphosate showed no effect on plant-shoot P concentrations and did not affect the root-mycorrhizal complex's ability to uptake P even in the presence of plant-limiting soil-P.

In conclusion, P fertilization to a low-P soil did have an effect on plant-shoot P concentrations for three species of RR agricultural crops. For mycorrhizal infection rates, P fertilization had a negative effect on RR soybeans only. Glyphosate had no effect for all treatments and all crops tested. Based on our data, we reject the hypothesis that glyphosate will decrease mycorrhizal infection rates. Implications from this study suggest that detrimental effects on mycorrhizal infection rates due to applications of glyphosate to RR soybean, corn, or cotton do not need to be taken into consideration for soil-P recommendations in low-P silt loam soil.

ACKNOWLEDGMENTS

The author thanks Drs. Savin and Purcell and the Dale Bumpers College of Agricultural, Food and Life Sciences.

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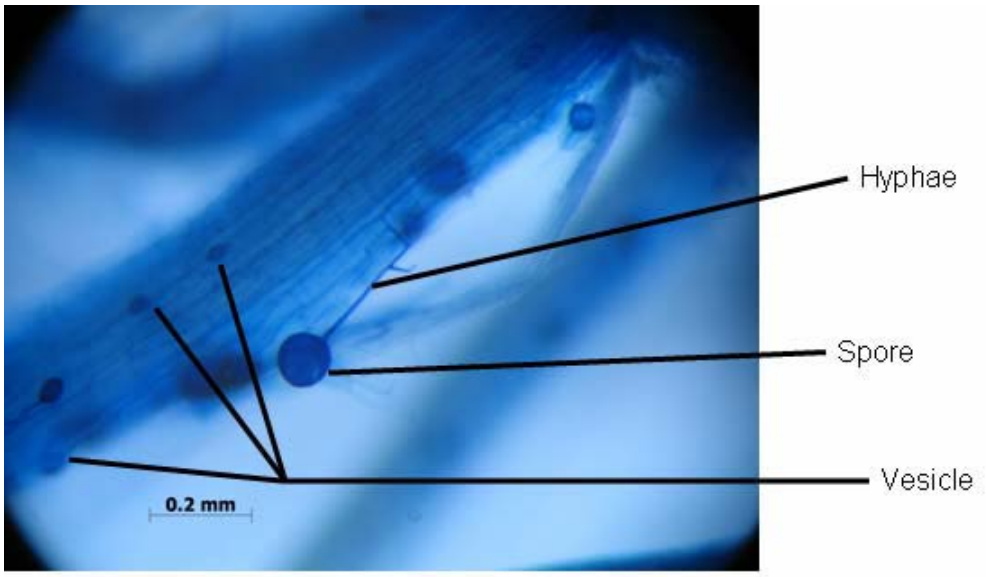

Fig. 1. Mycorrhizal infection of cotton root. Vesicular-arbuscular mycorrhizae can be identified using physical characteristics with the aid of a microscope. Vesicles can be identified as small oval pouches found within the plant root's outer cortex. Spores can be identified as an external circular entity attached to the end of fungal hyphae extending from the root's outer cortex.

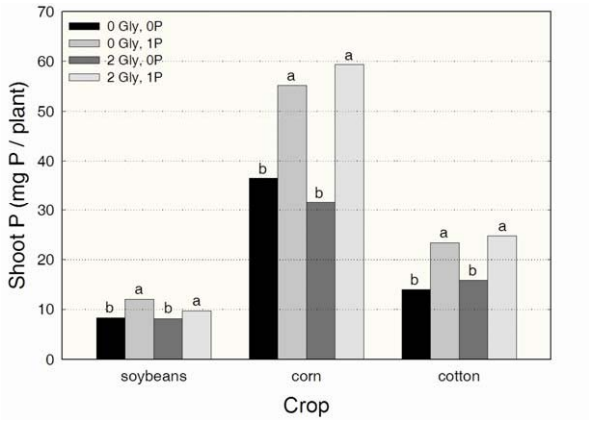

Fig. 2. Soybean, corn, and cotton plant-shoot phosphorus concentration. Treatments included glyphosate (0Gly and 2 Gly) and/or phosphorus (0P and 1P). Different letters within a crop show significant differences, P<0.05.

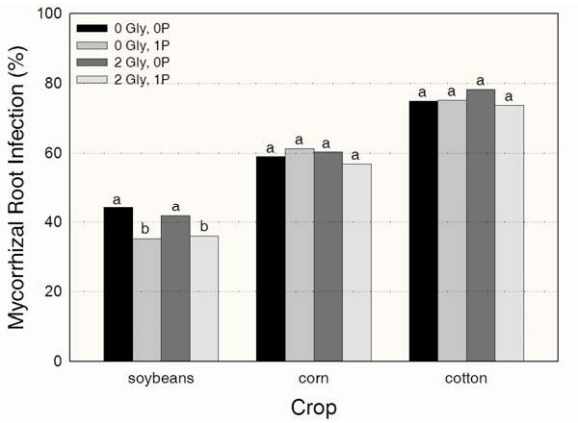

Fig. 3. Effect of treatment (glyphosate (0Gly and 2 Gly) and/or phosphorus (0P and 1P)) on mycorrhizal infection rates of soybean, corn, and cotton roots. Different letters within a crop show significant differences, P<0.05.

Table 1. Glyphosate and phosphorus applied to soybean, corn, and cotton plants

Treatment	Treatment symbols	Glyphosate (kg/ha)	P applied (kg P/ha)
1[z]	0Gly, 0P	0	0
2	0Gly, 1P	0	45
3	2Gly, 0P	1.1 x 2	0
4	2Gly, 1P	1.1 x 2	45

[z]Plants were exposed to 4 treatments: 1) no additional phosphorus (0P) or glyphosate (0Gly), 2) phosphorus (1P) and 0Gly, 3) 0P and glyphosate (2Gly), and 4) 1P and 2Gly. Glyphosate was applied twice at a rate of 1.1 kg/ha at about 10 and again at 20 days