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Effect of glyphosate on *Macrophomina phaseolina* in vitro and its effect on disease severity of soybean in the field☆

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ABSTRACT

Laboratory and field studies were conducted to assess the effects of glyphosate on *Macrophomina phaseolina* culture growth in vitro and the disease severity of charcoal rot in soybean fields at Stoneville, MS and Jackson, TN. Glyphosate inhibited *M. phaseolina* growth in a linear dose dependent manner when technical grade glyphosate acid (GlyCry) was used; however, growth was inhibited in an exponential dose dependent manner when a commercial formulation of glyphosate-potassium salt (Gly-K salt) was used. The glyphosate GR_{50} values (glyphosate concentration required to cause a 50% reduction) in culture radial growth ranged from 0.25 to 9.94 mM among the *M. Phaseolina* isolates, temperatures, and formulations. The three isolates differed in response to various concentrations across the three temperature regimes. Among the three isolates, TN 410 was the most sensitive for both GlyCry (GR_{50} = 7.74 mM) and Gly-K salt (GR_{50} = 0.25 mM) at 30 °C. This research indicates that glyphosate has the ability to inhibit growth of *M. phaseolina* in culture in vitro. The preliminary field studies demonstrated that application of glyphosate to glyphosate-resistant soybeans did not enhance or reduce the severity of charcoal rot in a no-till field in TN but had some suppressing effect in a tilled environment in MS when single applications were made at growth stage V3 and V6.

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1. Introduction

Charcoal rot of soybean, caused by *Macrophomina phaseolina* (Tassi) Goidanich, is one of the most important soilborne pathogens, infecting over 500 plant species in more than 100 plant families around the world (Smith and Wyllie, 1999). Charcoal rot has been a problem for soybean farmers in the United States for many years causing significant yield losses with estimated losses of 8.54×10^5 tonne per year from 1974 to 1994 in non-irrigated fields in the 16 southern states (Wrather et al., 2009; Wrather et al., 2006).

Symptoms of charcoal rot are also referred to as dry-weather wilt or summer wilt, because it often occurs when plants are under heat and drought stresses (Smith and Wyllie, 1999). These stresses can also occur in irrigated soybeans causing losses from 6 to 33% in experimental plots (Mengistu et al., 2011) and the combination of stress and the presence of *M. phaseolina* caused higher yield loss on soybeans than drought alone. The pathogen attacks the plant throughout the season, often causing progressive debilitation of the host. After flowering, a light gray or silvery discoloration of the epidermal and sub-epidermal tissues develops in the taproot and the lower part of the stem. The best diagnostic symptom is found when the epidermis is peeled away from the stem exposing numerous small, black bodies of microsclerotia that are frequently produced in the xylem and pith of the stem and may block water flow. Efforts to manage charcoal rot in soybean through adjusting planting dates, crop rotation, planting densities, and irrigation have all been suggested as means of control (Mengistu et al., 2007) and no commercial resistant soybean variety is yet available for effective management of this disease.

Glyphosate (N-[phosphonomethyl]glycine) application on glyphosate-resistant crops has been shown to enhance and in a few

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cases reduce severity (Johal and Huber, 2009) of selected soybean diseases. Glyphosate is widely used in glyphosate-resistant (GR) crops for weed management (Johal and Huber, 2009). Glyphosate is a systemic broad-spectrum herbicide that inhibits 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway for biosynthesis of aromatic acids and secondary metabolites (Means and Kremer, 2007). EPSPS is present in plants, fungi, and bacteria, but not in animals (Kishore and Shah, 1998). Blockage of this pathway results in massive accumulation of shikimate in affected plant tissues leading to a deficiency of significant end-products such as lignins, alkaloids, and flavonoids and a decrease in CO_2 fixation and biomass production in a dose dependent manner (Olesen and Cedergreen, 2010). Widespread use of glyphosate has raised a concern about its potential to affect plant pathogens in general and evolution of glyphosate-resistant weeds (Johal and Huber, 2009). Interactions between glyphosate use, other herbicides and plant diseases are well documented, with both positive and negative responses (Altman and Campbell, 1977; Johal and Huber, 2009). Glyphosate herbicide is known to increase specific plant diseases caused by pathogens such as *Corynespora cassiicola* (Huber et al., 2005), *Fusarium solani* f. sp. *glycines* (Johal and Huber, 2009), *Phytophthora megasperma* (Keen et al., 1982), *Heterodera glycines* (Geisler et al., 2002) and on micronutrient availability (Evans et al., 2007, Huber et al., 2004). An increase in colonization of GR soybean roots by *Fusarium virguliforme*, the causal agent of sudden death syndrome (SDS) of soybean showed that susceptibility was independent of the GR trait or glyphosate use (Nijiti et al., 2003; Sanogo et al., 2000; Sanogo et al., 2001). Kremer and Means (2009) reported that fungal colonization of GR soybean roots increased significantly after application of glyphosate but not after conventional post-emergence herbicides. Also, in studies examining effects of glyphosate on *Rhizoctonia* and *Sclerotinia* rots in GR crops, none demonstrated increased disease levels relative to untreated controls (Bradely et al., 2002; Harikrishnan and Yang, 2001; Pankey et al., 2005). Studies with GR wheat (*Triticum aestivum* L.) have shown that glyphosate provided both preventive and curative activities against *Puccinia striiformis* f. sp. *tritici* and *Puccinia triticina*, which cause stripe and leaf rusts, respectively (Feng et al., 2005). Preliminary greenhouse studies by Feng et al. (2005) reported that application of glyphosate in GR soybeans suppressed Asian soybean rust, caused by *Phakopsora pachyrhizi*. Analyses of GR soybean root exudates suggest that promotion of rhizosphere and root colonization of GR soybean by specific microbial groups may be due to a combination of stimulation by glyphosate released through root exudation and altered physiology leading to exudation into the rhizosphere of high levels of carbohydrates and amino acids (Kremer et al., 2005).

Glyphosate may be applied multiple times in commercial fields depending on field history, planting dates, environmental conditions and weed densities (Couler and Nafziger, 2007; Caleb et al., 2004). As a result, there is limited knowledge whether single or sequential applications of glyphosate in the field affect charcoal rot severity. Most of the research examining the effect of herbicides, including glyphosate, on disease development in soybean has been limited to greenhouse and laboratory studies (Anderson and Kolmer, 2005; Feng et al., 2005) and did not include application timing under different environments (Harikrishnan and Yang, 2001; Meriles et al., 2006). To test if glyphosate has any effect on *M. phaseolina*, it is necessary to conduct both in vitro and field studies. This study reports the effect of glyphosate on *M. phaseolina* in vitro and the effect of glyphosate on the population dynamics of *M. phaseolina* (colony forming units) collected from infected soybean in the field. Results of this study will help soybean growers determine whether glyphosate application on GR soybean under different environments may or may not increase the risk of charcoal rot in infested fields.

2. Materials and methods

2.1. Experiment 1. Effect of glyphosate on M. phaseolina growth in vitro

The treatments included six levels of concentrations of 0–20 mM of technical grade glyphosate acid (>97% purity, Sigma–Aldrich, St. Louis, MO) and twenty one levels of concentrations of 0–90 mM of the glyphosate-potassium salt formulation (48.8% glyphosate, N-(phosphonomethyl) glycine and 51.2% of other ingredients, Monsanto, St. Louis, MO). The treatments also included three *M. phaseolina* cultures; TN 4, TN 294 and TN 410 for evaluation of their growth in vitro. The three isolates were cultured from field collected samples in Jackson, TN and commonly used for routine field and greenhouse studies (data not published). Glyphosate stock solutions were prepared and filter sterilized. Glyphosate stocks were added to sterile molten potato dextrose agar (PDA, Difco Laboratories) to attain concentrations of 0–20 mM for pure glyphosate acid (GlyCry) and 0–90 mM for glyphosate-potassium salt (Gly-K salt). The solutions were mixed well, poured into 9-cm-diameter petri dishes, and the media was allowed to solidify. Using a 2-mm sterile cork borer, 7 day old cultures grown on acidified PDA at 30 °C were removed and placed at the center of five replicate plates and incubated at 24 °C, 28 °C, or 30 °C in the dark. Radial growth was recorded in four directions for each plate over the course of 72 h.

2.2. Experiment 2. Effect of glyphosate on charcoal rot severity on soybean in the field

Field studies were conducted in 2009 and 2010 at the USDA-ARS Crop Production Systems Research farm, Stoneville, MS, and at the West Tennessee Research and Education Center at the University of Tennessee in Jackson, TN both under non-irrigated environments. At both test locations, glyphosate treatments were: 1) glyphosate at 0.84 kg a.i/ha applied at V3 (third trifoliate); 2) glyphosate at 0.84 kg a.i/ha applied at V6 (sixth trifoliate); 3) glyphosate at 0.84 kg a.i/ha applied twice at V3 and V6; and 4) a no glyphosate applied (control). The commercial formulation of the potassium salt of glyphosate (Roundup WeatherMax, Monsanto Agricultural Co., St. Louis, MO) was used. Glyphosate treatments were applied with a CO_2-pressurized backpack sprayer that delivered 140 L/ha of spray solution at 193 kPa. All plots were hand weeded periodically throughout the growing season. No nitrogen fertilizer was applied, and the crop was not irrigated. Treatments were arranged in a randomized complete block design with four replications.

The field in Stoneville, MS had Dundee silt loam soil (fine-silty, mixed, active, thermic typic endoqualf) with pH 6.5, 1.0% organic matter and soil textural fractions of 26% sand, 55% silt, and 19% clay. The experimental area was in GR soybean production for three years prior to this study. The land was tilled with a disk harrow followed by a field cultivator in the fall of each year. A charcoal rot susceptible cultivar, GR soybean cultivar 'AG4605RR, late maturity group (MG) IV was planted 18 May 2009 and 28 April 2010 using a Monosem NG Plus precision planter (Monosem ATI, Inc. Lenexa, Kansas) at 285,000 seeds/ha. A tank mix of *S*-metolachlor at 1.12 kg a.i/ha plus pendimethalin at 1.12 kg a.i/ha plus paraquat at 0.84 kg a.i/ha was applied to the entire experimental area for early-season weed control immediately after planting with a tractor-mounted sprayer with TeeJet 8004 standard flat spray nozzles (TeeJet Spraying Systems Co., Wheaton, IL), at 187 L/ha water with 179 kPa. Each treatment plot consisted of eight rows spaced 51-cm apart and 7.6 m long.