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The interaction of two potential fungal bioherbicides and a sub-lethal rate of glyphosate for the control of shattercane

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Abstract

Greenhouse and laboratory experiments were conducted with the potential bioherbicides *Colletotrichum graminicola* (Cg) and *Gloeocercospora sorghi* (Gs) for control of shattercane weed. Single-spray tank mixture applications containing different ratios of the two fungi resulted in additive percent weed biomass losses. Intraspecific (Cg + Cg or Gs + Gs) and interspecific (Cg + Gs or Gs + Cg) sequential applications 1- or 7-days apart indicated antagonistic interactions in percent biomass loss. Application of either fungus with, or 1–3 days prior to, a sub-lethal concentration of glyphosate resulted in an antagonistic percent biomass loss; while application of glyphosate prior to either potential bioherbicide resulted in a synergistic weed disease response. Conidia germination studies conducted both *in vitro* on agar plates and with leaf impression peels suggest that antagonistic interactions observed in weed disease severity are probably due to the host–pathogen response following infection.

Keywords: *Colletotrichum graminicola*; *Gloeocercospora sorghi*; Pathogen mixture; *Sorghum* spp.; Colby's equation; Antagonistic; Additive; Synergistic; Integrated weed management

1. Introduction

Shattercane [*Sorghum bicolor* (L.) Moench.) ssp. *drummondii* (Nees ex Steud.) de Wet ex Davidse] is a large, annual grass that propagates by seeds (Horak and Moshier, 1994). The exact ancestry is unknown, but it probably originated from natural crosses of forage sorghums with other sorghum species in southern and eastern Africa. It has become well established in agricultural areas ranging from temperate to tropical regions throughout the world. This weed reduces crop yields (Tarr, 1962), hosts insect and disease pests of grain sorghum [*S. bicolor* (L.) Moench.] (Forrester et al., 1975; Tarr, 1962) and hybridizes with grain sorghum (Defelice, 2006).

Chemical control of weeds is likely to face limitation in the future because of concern for the environment. Herbicides are the most effective immediate solution to most weed problems, but other solutions including bioherbicides are becoming available (Hallett, 2005). The Multistate Research Project S-1001, sanctioned by the USDA-CSREES, is a current collaborative effort supported by many scientists with the goal of identifying indigenous plant pathogens that may be used as biological herbicides.

Only a few examples have been documented where investigators improved weed control either by using a combination of bioherbicides or combining a bioherbicide with a chemical herbicide in an integrated pest management (IPM) approach. Morin et al. (1993) demonstrated synergy between *Puccinia xanthii* Schw. and *Colletotrichum orbiculare* (Berk. and Mont.) v. Arx for the control of Noogoora burr (*Xanthium occidentale* Bertol.). Following inoculation after infection by the rust fungus, *C. orbiculare* spread necrotrophically in rust lesions. This resulted in severe disease and sometimes death of weeds if originating from stem lesions. *Pseudomonas* species in the phylloplane reportedly

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enhanced disease caused by *Colletotrichum coccodes* (Wallr.) Hughes on velvetleaf [*Abutilon theophrasti* Medic.] (Fernando et al., 1994). The hypothesis is that bacteria stimulate formation of appresoria which subsequently accelerates infection by *C. coccodes*. Chandramohan and Charudattan (2003) used a multiple pathogen mix for effective control of three broadleaf weeds. Smooth pigweed (*Amaranthus hybridus* L.), showy crotalaria (*Crotalaria spectabilis* Roth.) and sicklepod (*Senna obtusifolia* (L.) Irwin and Barneby) seedlings were inoculated with a mixture of four fungal pathogens targeting these weeds. All weeds were killed within 6 weeks without loss of efficacy or alterations in host specificity of each fungus in the mixture. Brooker et al. (1996) combined the natural tripeptide herbicide bialaphos (L-phosphinothricyl-L-alanine-L-alanine) with a strain of *Colletotrichum gloeosporioides* (Penz.) Sacc. f.sp. *aeschynomene* genetically engineered for resistance to this herbicide for control of both northern (*Aeschynomene virginica* (L.) Britton, Sterns and Poggenb) and Indian (*Aeschynomene indica* L.) jointvetch. Coapplication of the bioherbicide with bialaphos resulted in a synergistic effect on disease severity for both weed species. Tank mixtures of two *Bipolaris* spp. with metolachlor [2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxyl-methyl-ethyl)acetamide] were used successfully to control johnsongrass in growth chamber and field tests (Winder and Van Dyke, 1990). Grant et al. (1990) evaluated control of round-leaved mallow (*Malva pusilla* Sm.) using several herbicides in tank mix and split applications with the bioherbicide *Colletotrichum gloeosporioides* (Penz.) Sacc. f.sp. *malvae*. Several combinations (dependent upon timing) were found to enhance round-leaved mallow control over the bioherbicide alone. Smith and Hallett (2006) demonstrated that half the recommended label rate of glyphosate (0.315 kg/ha) was needed for control of common waterhemp (*Amaranthus rudis* Sauer) when combined with *Microsphaeropsis amaranthi* (Ell. and Barth.) inoculated within 1–3 days of herbicide treatment.

Anthracnose, caused by *Colletotrichum graminicola* (Ces.) Wils., and zonate leafspot, caused by *Gloeocercospora sorghi* D. Bain and Edg., are common diseases of cereals and grasses, including shattercane. Mitchell (1993a) evaluated host specificity of johnsongrass (*Sorghum halepense* (L.) Pers.) isolates of these two fungi against *S. bicolor* (22 cultivars), *S. sudanense* Stapf. (2 cultivars), *S. bicolor* × *S. sudanense* (2 cultivars) and 27 cultivars of *Zea mays* L. Results demonstrated that these two fungi are too virulent to be used as bioherbicides in grain sorghum (*S. bicolor*) and Sudan grass (*S. sudanense*) but may be used to control johnsongrass in dicotyledonous crops. Chiang et al. (1989) also tested these fungi as potential bioherbicides and recommended they not be used in corn and sorghum. Our tests demonstrated that 11 sweet and 16 dent corn cultivars exhibited hypersensitive flecking but plants grew out of this symptom within 10 days. *Gloeocercospora sorghi* was more virulent against johnsongrass over a wider range of host ages (1–5 leaf stage), dew temperatures (15–30 °C) and inoculum densities (1×10^5–1×10^7 conidia/ml) than *C. graminicola* in greenhouse trials (Mitchell, 1993a). Growth chamber experiments demonstrated that post-inoculative air temperatures ranging 25–35 °C had no affect on disease severity if johnsongrass plants were treated with *G. sorghi* and *C. graminicola* concentrations of 1×10^6 and 1×10^7 conidia/ml or greater, respectively (Mitchell, 1993b). A mathematical model derived from dew chamber studies was highly correlated with field results and was successfully used to predict johnsongrass damage that occurred (Mitchell, 1993b). In one field trial 91% biomass loss resulted in johnsongrass infected with *G. sorghi*.

The primary goals of this study were to determine: (1) if there is a synergy when using these two bioherbicide candidates either in tank mix or split applications and (2) if control of shattercane can be enhanced by combining either of these fungi with a sub-lethal rate of glyphosate.

2. Materials and methods

2.1. Fungal bioherbicide candidates

Isolates of *C. graminicola* (A37) and *G. sorghi* (GL1) were collected from sporulating lesions on johnsongrass growing within Bexar County, TX. Mitchell (1993a) demonstrated that isolates of these two fungi also cause disease in *S. bicolor* and *S. sudanense*. Pure cultures were obtained by aseptically removing acervuli and sporodochia, respectively, with a sterile dissecting probe, placing onto a potato-dextrose agar plate and streaking for isolation with a double-wired inoculating loop. Stock cultures were maintained in 5 percent w/v non-fat dry skim milk and 20 percent v/v glycerol at −80 °C and deposited at the University of Arkansas (Plant Pathology Department). Inoculum of *G. sorghi* was produced by transferring aliquots from stock cultures onto 23 percent v/v unsalted sweet pea brine agar (1.8 percent w/v) Petri plates (Mitchell et al., 2003). Plates were incubated on the lab bench at room temperature (23–24 °C) for 6 days under fluorescent lights (1:1, Gro-Lux:-Cool White) positioned 35 cm above the plates and adjusted to a 14-h photoperiod (320 Lumen/cm^2). Inoculum of *C. graminicola* was produced in a similar fashion on M100 medium: 15 g Maltrin M100 (Grain Processing Corporation, Muscatine, IA), 15 g Provesta Savory 001 (Pure Culture Products, Chicago, IL), 1 g K_2HPO_4, 0.5 g $MgSO_4 \times 7H_2O$ and 15 g agar per 1 L deionized water. Conidia were suspended in deionized water with a cotton swab and washings were filtered through four layers of cheesecloth.

2.2. Shattercane plants

Seeds of shattercane were obtained from Steven Hallett, Ph.D. (Purdue University). Test plants were grown on greenhouse benches at 25–30 °C in 7.5 × 9 cm plastic pots