Biocontrol Science and Technology, 2006; 16(10): 1067–1077

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Glyphosate and bioherbicide interaction for controlling kudzu (*Pueraria lobata*), redvine (*Brunnichia ovata*), and trumpetcreeper (*Campsis radicans*)

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(Received 27 January 2004; returned 24 February 2004; accepted 10 April 2006)

Abstract

In controlled environment experiments, the bioherbicidal fungus *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar:Fr. was tested alone, in combination with, prior to, and following treatment with glyphosate [*N*-(phosphonomethyl)glycine] for control of kudzu [*Pueraria lobata* (Willd.) Ohwi], redvine [*Brunnichia ovata* (Walt.) Shinners], and trumpetcreeper [*Campsis radicans* (L.) Seem. ex Bureau] at temperatures of 20, 30, and 40°C. At all temperatures, kudzu was most adversely affected by the fungus, followed by trumpetcreeper and redvine, as indicated by greater mortality and dry weight reductions. Trumpetcreeper and redvine mortalities and dry weight reductions significantly increased when the fungus was applied 2 days after the glyphosate treatment. Application of the fungus combined with or prior to glyphosate treatment resulted in reduced weed control. Although pathogenesis and mortality also occurred at 20°C, disease development was favored by higher temperatures (30 and 40°C). Infected weeds of each species exhibited similar disease symptomatology within 12 h following treatment at incubation temperatures of 30 and 40°C. Disease symptomatology was characterized by necrotic flecking on leaves that coalesced into large lesions. Symptoms progressed, initially infecting cotyledons and leaves, and later (within 48 h) producing stem lesions. The fungus sporulated profusely on infected tissue and was easily reisolated. These results suggest that timing of glyphosate application in relation to combined treatment with the bioherbicide *M. verrucaria* can improve the control of kudzu, redvine, and trumpetcreeper.

Keywords: Myrothecium verrucaria, *biological control, bioherbicide, kudzu,* [Pueraria lobata *(Willd.) Ohwi*], *redvine,* [Brunnichia ovata *(Walt.) Shinners*], *synergistic interaction, trumpetcreeper,* [Campsis radicans *(L.) Seem. ex Bureau*]

Introduction

Kudzu [*Pueraria lobata* (Willd.) Ohwi] is a perennial leguminous vine native to eastern Asia. Kudzu was introduced into the US in the late 1800s (McKee & Stephens 1943) and presently occurs from Florida to New York, westward to central Oklahoma and Texas, with the heaviest infestations in Alabama, Georgia, and Mississippi (Miller 1996). It was cited in a report by Congress in 1993 as one of the most harmful non-indigenous plant species in the US, and was listed as a federal noxious weed in 1998.

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ISSN 0958-3157 print/ISSN 1360-0478 online
DOI: 10.1080/09583150600828742

Redvine and trumpetcreeper are common perennial vines found in cultivated and reduced tillage fields, wastelands, fence rows, and riverbanks in the lower Mississippi River alluvial plain and are among the 10 most troublesome weeds in row crops grown in this geographical region (Elmore 1984). These weeds reduce crop yield and quality, and interfere with cultivation and harvest operations.

Redvine and trumpetcreeper are difficult to control because of their extensive deep root systems (Elmore 1984; Chachalis & Reddy 2000; Chachalis et al. 2001). Many herbicides with potential to control these weeds kill only the foliage, with little or no translocation to rootstock (Shaw & Mack 1991; Chachalis & Reddy 2000). Glyphosate, a widely used, nonselective broad-spectrum postemergence herbicide exhibits some activity on redvine (Chachalis & Reddy 2000; Chachalis et al. 2001; Reddy & Chachalis 2004). Glyphosate at $1-2\times$(1.1 and 2.2 kg ha^{-1}, respectively) use rates provide 60–90% control of these weeds, but plants reestablish in within 4–6 weeks after treatment. Regrowth from underground rootstocks occurs because of insufficient translocation of glyphosate to rootstocks (Reddy 2000; Reddy & Chachalis 2004). Thus, destruction of foliage is only temporary and new sprouts arise from the rootstocks.

The fungus *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar:Fr., originally isolated from sicklepod (*Senna obtusifolia* (L.) Irwin & Barneby] exhibited excellent biocontrol potential for several weed species, including the legumes sicklepod and hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill], when formulated with the surfactant Silwet L-77 (a silicone-polyether copolymer spray adjuvant, OSi Specialties, Inc., Charlotte, NC) (Walker & Tilley 1997; Andersen & Hallett 2004). This fungus also effectively controlled kudzu in the absence of a dew treatment over a wide range of physical and environmental conditions in the greenhouse and under field conditions (Boyette et al. 2002). Redvine and trumpetcreeper were not examined as potential hosts in any of these reports, and these weeds have not been previously reported in the literature as hosts of *M. verrucaria.* Preliminary experiments revealed that both redvine and trumpetcreeper were modestly susceptible to *M. verrucaria*, but to a degree that would not provide acceptable weed control (Boyette et al. 2001). Previous research has shown that it is possible to significantly improve the performance of several bioherbicides through chemical synergism, especially with glyphosate (Boyette & Hoagland 2000; Hoagland 2000; Hoagland et al. 2001). The objectives of this present study were to determine the effects of *M. verrucaria*/glyphosate mixtures, sequential applications, and post-inoculation temperatures on mortality and dry weight reduction of kudzu, redvine, and trumpetcreeper. Furthermore, we wished to classify possible combined effects of glyphosate and this bioherbicide as additive, antagonistic, or synergistic interactions.

Material and methods

Inoculum production

Inocula (spores) of *M. verrucaria* for all experiments were produced in Petri dishes containing Difco (Difco Laboratories Inc., Detroit, MI 48232, USA) potato dextrose agar (PDA). Agar surfaces were flooded with 3 mL of a *M. verrucaria* conidia suspension containing 2×10^6 conidia mL^{-1}. The PDA plates were inverted on open-mesh wire shelves and incubated at 25°C for 5 days in fluorescently lighted incubators.

The resulting conidia were rinsed from the plates with sterile, distilled water, and were adjusted to the required concentrations by adding distilled water. Spore counts and concentrations were estimated with hemacytometers.

Test plant propagation and treatments

Kudzu seedlings were grown from seed (obtained from Adams-Briscoe Seed Co., Jackson, GA 30233, USA) that were placed on moistened filter paper contained in Petri dishes, and incubated at 28°C for 3 days in a darkened incubator to induce germination. Redvine and trumpetcreeper seed were collected in the fall of 1999 near the SWSRU farm, Stoneville, MS. These seeds were germinated in Bosket sandy loam prior to planting. Germinated seeds of the three weed species were then planted separately in 10-cm plastic pots (one seedling per pot) containing a 1:1 commercial potting mix (Jiffy Products of America, Inc., Batavia, IL 60510, USA)/sandy loam soil combination that was supplemented with a controlled-release (14:14:14, NPK) fertilizer (Grace Sierra Horticultural Products, Milpitas, CA 95035, USA) and placed on greenhouse benches. The plants were sub-irrigated daily. Temperatures in the greenhouse ranged from 28 to 32°C with 40–60% relative humidity. The photoperiod was approximately 14 h, with 1600–1800 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) at midday, as measured with a light meter.

Kudzu, redvine and trumpetcreeper plants in the second- to fourth-leaf stages of growth (10–14 days old) were inoculated by spraying until the foliage was fully wetted (ca. 100 L ha^{-1}) with suspensions containing 2×10^7 conidia mL^{-1} plus 0.02% Silwet L-77 surfactant (2×10^{12} conidia ha^{-1}). Control plants were sprayed with 0.02% surfactant only. Hand-held aerosol sprayers (Spra-Tool, AERVOE Industries, Gardnerville, NV 89401, USA) were used to make all applications. New aerosol canisters were used to deliver high and equal pressure (and volume delivery) to all treatment sets. Treatments consisted of: (1) glyphosate only (GLY); (2) *M. verrucaria* only (MV); (3) GLY followed by (fb) MV 2 days after treatment (DAT); (4) MV fb GLY 2 DAT); (5) MV+GLY; and (6) untreated (UNT). Glyphosate was applied at a rate of 1.1 kg ha^{-1}. Experimental units consisted of groups of 10 plants and the treatments were replicated four times. Immediately following inoculation, the plants were placed in Shearer growth chambers (Rheem Mfg. Co., Weaverville, NC 28787, USA) at constant day/night temperatures of 20, 30, or 40°C. Photoperiods were 12 h day/12 h night with approximately 900 μmoL m^{-2} s^{-1} PAR. Disease development was monitored daily, and 14 days after inoculation all 10 plants of each experimental unit, both living and dead, were excised at the soil line, combined, and dried (80°C for 7 days) for dry-weight determinations.

Experimental design

The experiments were arranged as randomized complete block designs, were repeated in time, and the data within each temperature regimen for each experiment were averaged following subjection to Bartlett's test for homogeneity of means (Steele & Torrey 1980). Means within each temperature regimen were separated using Fisher's least significant difference at $P = 0.05$.

Statistical tests for treatment interactions

To differentiate herbicide–pathogen (glyphosate–MV) interactions, a method based on a multiplicative survival model was used (Colby 1967). Colby's formula ($E = AB/100$) was used to obtain expected mortality values (E) if responses were additive. Paired t-tests compared the expected mortality (E) with the observed mortality for combined chemical (A = glyphosate) and pathogen (B = MV) treatments to determine if there were significant synergistic or antagonistic responses. For the t-test, the variance of the treatment means was obtained ($P \leq 0.05$), using the ANOVA procedure of SAS (SAS Institute 1989). Colby's expected mortality is a function of two of the treatment means (A and B), and this formula includes the product of these two means. An approximation for the variance of the product of two random variables was used to obtain estimated variance for Colby's expected mortality. Significant differences in treatment means were obtained at all three temperature regimes. However in practice, the application of herbicide and bioherbicide would be most appropriately performed on these weed species at moderate temperatures (e.g. 25–35°C), hence only the 30°C data were analyzed for herbicide–pathogen interactions.

Results and discussion

Pathogenesis and mortality occurred at all temperatures that were tested; however, for all weed species, greater weed control and dry weight reductions occurred at 30°C than at 20 or 40°C (Figures 1–6). Disease symptoms progressed after inoculation from infected cotyledons and leaves, to stem lesions within 48 h. Kudzu was controlled more efficaciously than either redvine or trumpetcreeper at 30 and 40°C

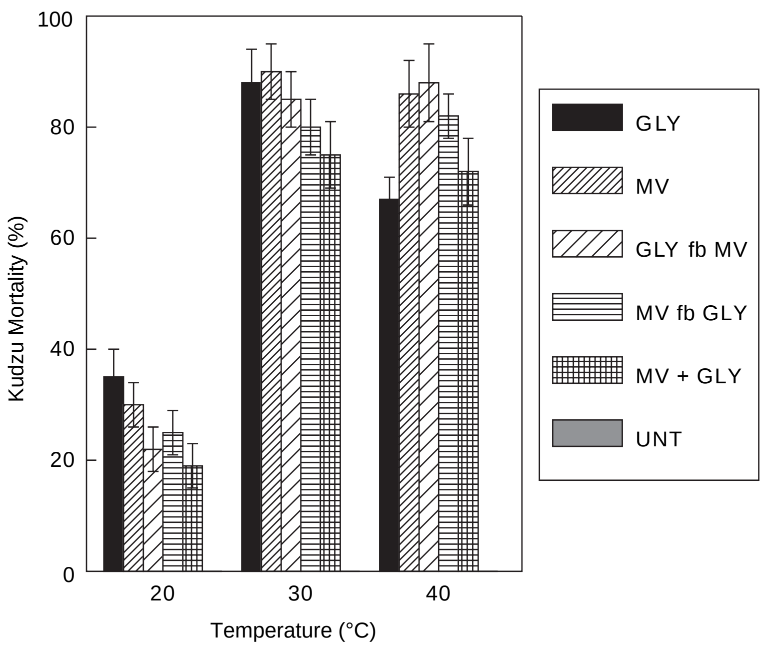

Figure 1. Effects of *Myrothecium verrucaria*, glyphosate, and temperature on mortality of kudzu. Vertical lines represent Fisher's protected least significance test values at $P = 0.05$.

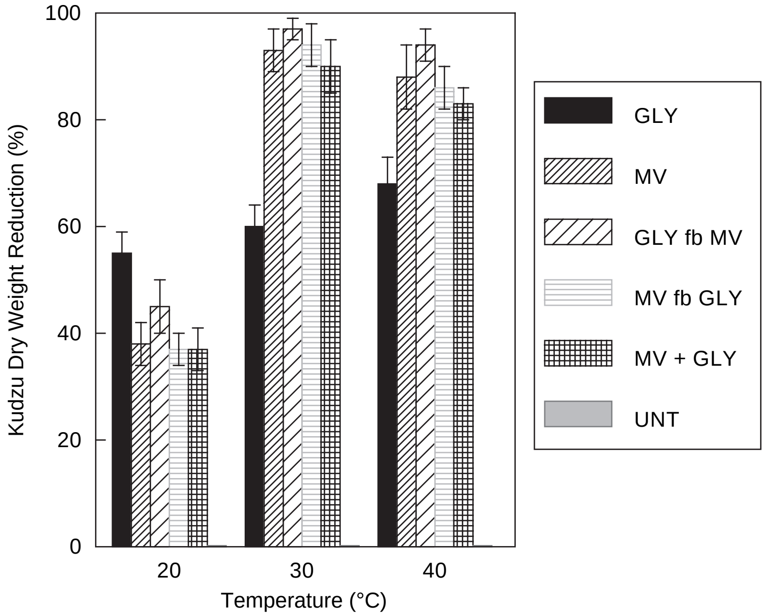

Figure 2. Effects of *Myrothecium verrucaria*, glyphosate, and temperature on dry weight reduction of kudzu. Vertical lines represent Fisher's protected least significance test values at $P = 0.05$.

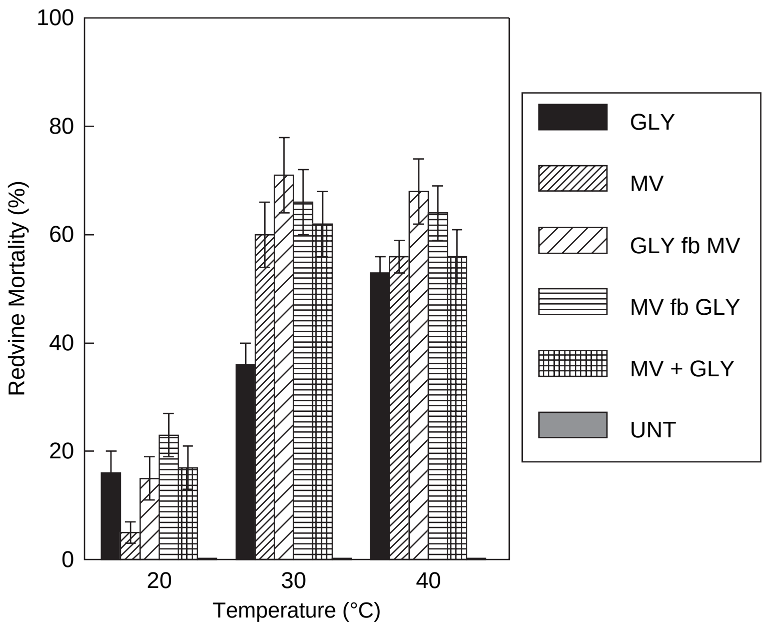

Figure 3. Effects of *Myrothecium verrucaria*, glyphosate, and temperature on mortality of redvine. Vertical lines represent Fisher's protected least significance test values at $P = 0.05$.

regardless of spray treatment. The control of kudzu and redvine achieved with the bioherbicide alone treatment was significantly greater than that treated with glyphosate alone (Figures 1, 3, and 5). Control of all weed species was significantly

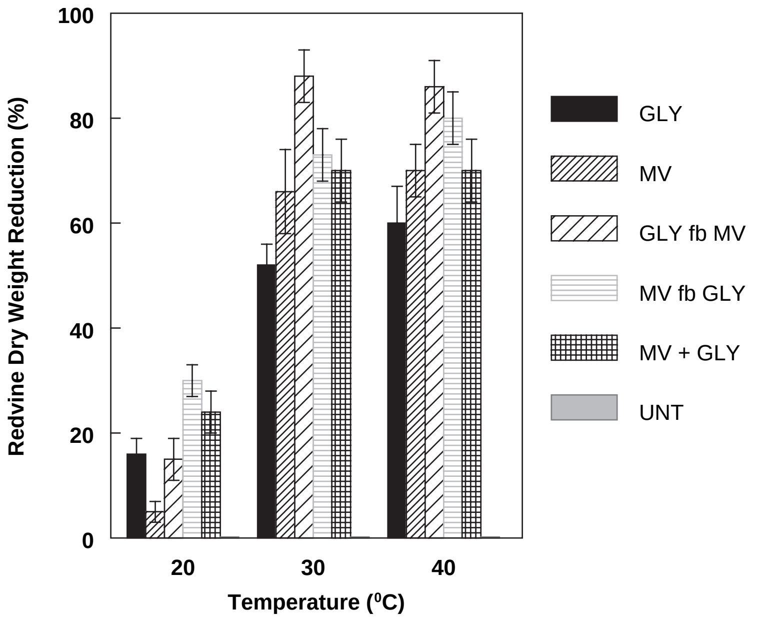

Figure 4. Effects of *Myrothecium verrucaria*, glyphosate, and temperature on dry weight reduction of redvine. Vertical lines represent Fisher's protected least significance test values at $P = 0.05$.

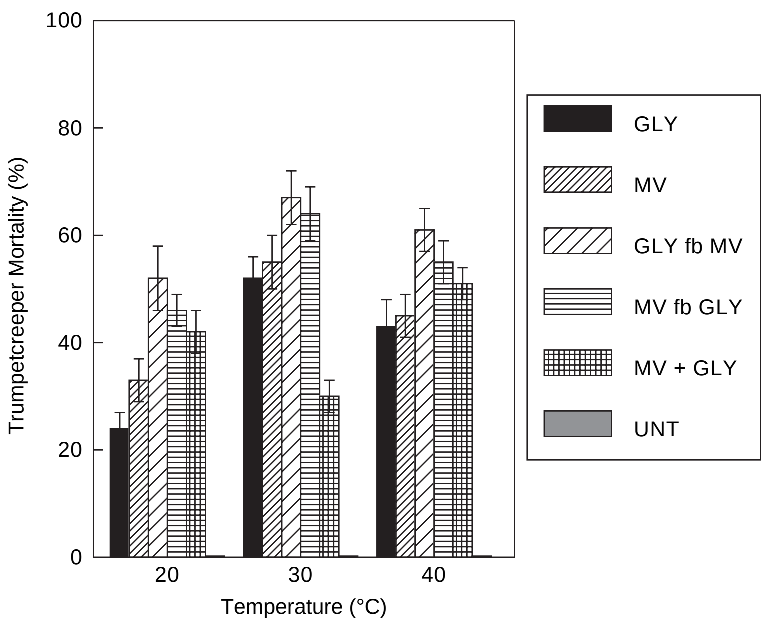

Figure 5. Effects of *Myrothecium verrucaria*, glyphosate, and temperature on mortality of trumpetcreeper. Vertical lines represent Fisher's protected least significance test values at $P = 0.05$.

less at 20°C (Figures 1–6). Control and dry weight reductions of redvine and trumpetcreeper were significantly higher when glyphosate was applied prior to the bioherbicide treatment (Figures 3–6). Control was reduced when the bioherbicide

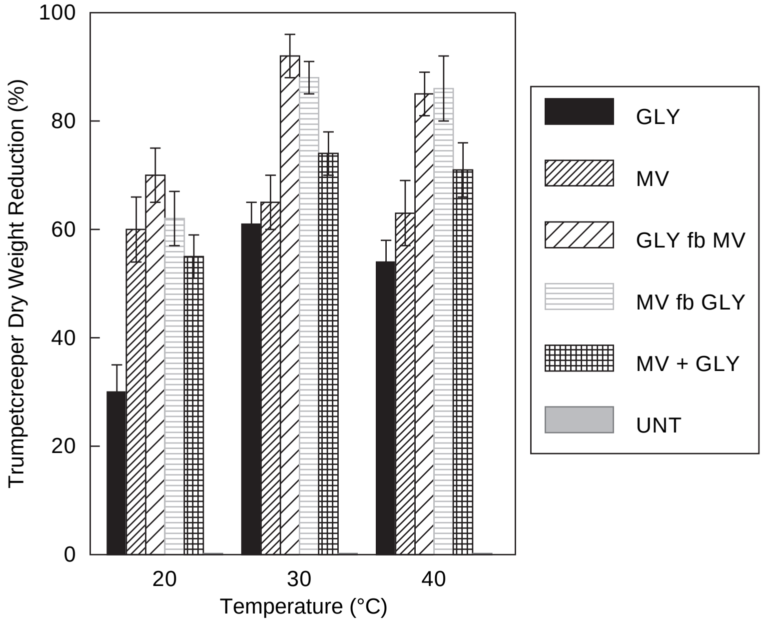

Figure 6. Effects of *Myrothecium verrucaria*, glyphosate, and temperature on dry weight reduction of trumpetcreeper. Vertical lines represent Fisher's protected least significance test values at $P = 0.05$.

was applied prior to glyphosate and when the bioherbicide and pathogen were tank mixed (Figures 1, 3, and 6).

Overall, these experiments revealed that redvine and trumpetcreeper were more tolerant than kudzu to either glyphosate or MV at the rates tested here. Interaction analyses revealed that no interaction occurred between glyphosate and MV for kudzu control with any application treatment at 30°C (Table I). A synergistic effect occurred when MV was applied 2 days after glyphosate application to redvine (Table I). Additive effects occurred when glyphosate was applied either 2 days before, or 2 days after MV application, and when glyphosate and MV were applied simultaneously as a mixture to redvine (Table I). Additive effects also occurred when glyphosate was applied either 2 days before, or 2 days after MV application to trumpetcreeper. An antagonistic interaction was noted when glyphosate and MV were applied simultaneously to trumpetcreeper (Table I).

Although an antagonistic effect between glyphosate and MV was shown to exist in only a single treatment with only one weed species, weed mortality was significantly reduced in all species when glyphosate and MV were applied as mixtures. Whether these effects are due to the active ingredient *N*-(phosphonomethyl)glycine or to certain adjuvants contained in the formulated herbicide product (Rounup-Ultra™) is currently unknown. Additional studies will be conducted to further elucidate these findings.

Several reviews and reports have described various plant pathogen:herbicide interactions as additive, antagonistic, or synergistic. More specifically, interactions between various herbicides and plant pathogens (Hoagland et al. 1979; Altman et al. 1990; Smith 1991; Hoagland 1996, 2001), herbicide induction of microbial invasion of plant roots (Greaves & Sargent 1986), and the interactions of sub-lethal herbicide

Table I. Interactions of glyphosate and *M. verrucaria* for control of kudzu, redvine, and trumpetcreeper 14 days after application under 30°C growth conditions.

Treatment	Observed	Expected[a]	Interaction[b]
	%		
Kudzu			
Glyphosate	88	79	ns
M. verrucaria	90	79	ns
Glyphosate, *M. verrucaria*	85	75	ns
M. verrucaria, glyphosate	80	70	ns
Glyphosate+*M. verrucaria*	75	66	ns
Redvine			
Glyphosate	35	21	ns
M. verrucaria	60	21	ns
Glyphosate, *M. verrucaria*	70	24	Synergistic
M. verrucaria, glyphosate	65	23	Additive
Glyphosate+*M. verrucaria*	62	22	Additive
Trumpetcreeper			
Glyphosate	52	29	ns
M. verrucaria	55	29	ns
Glyphosate, *M. verrucaria*	67	35	Additive
M. verrucaria, glyphosate	65	34	Additive
Glyphosate+*M. verrucaria*	30	16	Antagonistic

[a]Expected values determined by the Colby equation: $E_1 = (X_1 Y_1)/100$, where E_1 is expected mortality with herbicides A+B, X_1 is observed mortality with A (glyphosate), and Y_1 is observed mortality with B1 (*M. verrucaria*). [b]Significantly different from observed value ($P \geq 0.05$) as determined by a t-test; non-significant interactions are denoted by ns.

doses on root pathogens (Lévesque & Rahe 1992) have been reviewed. One of the earliest reports indicating that herbicides could block resistance to pathogens was the increased infection of an incompatible race of *Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D. C. Erwin in soybean caused by glyphosate (Keen et al. 1982). Low levels of glyphosate reduced the phytoalexin glyceollin, which was suggested as the possible operative mechanism. Other synergistic interactions of *A. cassiae* and sicklepod with glyphosate showed that glyphosate suppressed sicklepod defense response by lowering phytoalexin [2-(*p*-hydroxyphenoxy)-5,7-dihydroxychrome] production (Sharon et al. 1992). Glyphosate could be acting in a similar fashion in kudzu, redvine and trumpetcreeper. Whether these effects are due to pathogen spore germination and growth interactions, or to effects on weed defenses is also a subject of future research in our laboratory.

Compatibility of bioherbicides with chemical pesticides and surfactants is an important factor in their overall utility and acceptance. For example, Wyss et al. (2004) demonstrated that certain pesticides and their adjuvants affected spore germination and vegetative growth of *Phomopsis amaranthicola* Rosskopf, Charudattan, Shabana, et Benny sp. nov., an effective bioherbicide against pigweeds (*Amaranthus* spp.) (Rosskopf et al. 2000a,b). Several herbicides and their adjuvants generally had a negative effect on spore germination of *Pyricularia setariae* Niskoda, a green foxtail [*Setoria viridis* (L.) Beauv.] pathogen (Peng & Byer 2005). One strategy to overcome direct toxicities of agrochemicals and their formulation additives is a sequential, rather than simultaneous, application of chemical and bioherbicide. Here we have demonstrated that in general, application of glyphosate prior to MV provided better weed control in these three weeds compared to simultaneous application, or application of the bioherbicide prior to glyphosate. Similarly, application of several herbicides 6 h or earlier, prior to pathogen (*P. setariae*) application to green foxtail, generally resulted in greater efficacy (Peng & Byer 2005). Furthermore, glyphosate and glufosinate [2-amino-4-(hydroxymethylphosphinyl)butanoic acid] interactions with this bioherbicide were found to be synergistic.

The commercial importance of the discovery of synergistic interactions of bioherbicides with agrochemicals has also been demonstrated resulting in the issuance of US patents (Caulder & Stowell 1988a,b). In these studies, the herbicides acifluorfen {5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid} and bentazon {3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide} were effective synergists that provided increased control in several weed:pathogen combinations, such as sicklepod:*Alternaria cassiae* Jurair & A. Khan, northern jointvetch [*Aeschynomene virginica* (L.) B.S.P.]:*Colletotrichum gloeosporioides*, and Florida beggarweed [*Desmodium tortuosum* (SW.) DC.]:*C. truncatum* (Schwein.) Andrus & Moore.

If weed defenses can be lowered, weeds should be more susceptible to disease. Numerous examples in the literature have correlated phenylalanine ammonia-lyase (PAL) activity with pathogen challenge and plant defense (Hoagland 1999). Compounds that inhibit PAL, in most cases, have caused increased susceptibility to disease. Furthermore, glyphosate and several other herbicides can alter the levels of PAL, phytoalexins, and phenolic compounds in plants, all of which affect plant defense against pathogens (Hoagland 2000). However, since most of these studies on secondary plant metabolism and plant defense mechanisms deal with crop plants, the role of these factors in weed:pathogen interactions merits further investigation.

Certain other weed pathogens have been discovered that might be more prone to commercialization if they prove to be compatible with registered agrochemicals (herbicides or plant growth regulators), or if their weed control efficacies could be increased via additive or synergistic interactions with such agrochemicals. These present results suggest that it is possible to enhance the bioherbicidal potential of *Myrothecium verrucaria* using herbicides such as glyphosate.

Acknowledgements

We thank J.R. McAlpine, A. Tidwell, and V. Robertshaw for valuable technical assistance. The advice and expertise provided by Debbie Boykin regarding statistical considerations of this data is greatly appreciated.

Note

1 Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by USDA-ARS and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

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