RESEARCH PAPER

Redvine (*Brunnichia ovata*) and trumpetcreeper (*Campsis radicans*) controlled under field conditions by a synergistic interaction of the bioherbicide, *Myrothecium verrucaria*, with glyphosate

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In field experiments conducted near Stoneville, MS, USA, in 2000 and 2001, the bioherbicidal fungus, *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar: Fr., was tested alone and in combination with a glyphosate (*N*-[phosphonomethyl]glycine) product for controlling natural infestations of the invasive vines, redvine (*Brunnichia ovata* [Walt.] Shinners) and trumpetcreeper (*Campsis radicans* [L.] Seem. ex Bureau). After 12 days, redvine and trumpetcreeper were controlled by 88% and 90%, respectively, through a synergistic interaction between the fungus and the herbicide, glyphosate. The disease symptomatology was characterized by rapid necrosis of the leaf and stem tissues, with mortality occurring within 72 h. Neither glyphosate alone, nor *M. verrucaria* alone, controlled these weeds at commercially acceptable levels (≥80%). No visual disease or herbicide damage occurred to the soybean in the treated test plots 12 days after planting. These results suggest that some formulations of glyphosate, mixed with *M. verrucaria*, can effectively control redvine and trumpetcreeper.

Keywords: bioherbicide, *Brunnichia ovata*, *Campsis radicans*, interaction, *Myrothecium verrucaria*.

INTRODUCTION

Redvine (*Brunnichia ovata* [Walt.] Shinners) and trumpetcreeper (*Campsis radicans* [L.] Seem. ex Bureau) are native perennial, deciduous, woody dicotolydenous, shrubby vines capable of growing several meters in length (Elmore 1984). These weeds are often found in dense populations in cultivated and fallowed fields, wastelands, fence rows, yards, river banks, swamps, and forests and are distributed extensively in the lower Mississippi Alluvial Plain area (Mississippi Delta) of the southern USA. These weeds are rated among the 10 most troublesome weeds in the row crops of the Mississippi Delta region as they reduce crop yield and quality and interfere with cultivation and harvest operations (Elmore 1984).

Glyphosate (*N*-[phosphonomethyl]glycine) has become the predominant postemergence herbicide used in soybean (*Glycine max* [L.] Merr.) in the USA, with ~80% of soybean acres sown to glyphosate-resistant varieties (Duke 2005). Glyphosate is a broad-spectrum postemergence herbicide with some herbicidal activity on redvine and trumpetcreeper (Chachalis & Reddy 2000; Chachalis *et al.* 2001; Reddy & Chachalis 2004). The control of these weeds with glyphosate alone, even at rates that are two-to-four times the rates recommended for non-genetically modified soybean, is temporary at best and, alone, cannot satisfactorily control these weeds. As alternatives to synthetic herbicides, plant pathogens have been evaluated as weed control agents (Charudattan 2001, 2005; Hoagland 2001; Boyetchko *et al.* 2002;

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Boyetchko & Peng 2004; Hallett 2005). The concept of combining microbial herbicides with chemical herbicides has been the subject of considerable research. Furthermore, it has been demonstrated that combinations of some bioherbicides and synthetic herbicides can be synergistic (Caulder & Stowell 1988; Christy *et al*. 1993), resulting from lowered weed defense responses caused by the herbicides, thus making the weeds more susceptible to pathogen attack (Hoagland 1996, 2000). Several reports indicated that coapplications of *Colletotrichum coccodes* Wallr. and the plant growth regulator, thidiazuron (*N*-phenyl-*N′*-1,2,3-thiadiazol-5-yl-urea), to velvetleaf (*Abutilon theophrasti* Medic.) increased pathogen infection and weed control compared with either component applied alone (Wymore *et al*. 1987; Hodgson *et al*. 1988; Wymore & Watson 1989). Heiny (1994) found that *Phoma proboscis* Heiny, at 1×10^7 spores mL^{-1} and applied with 2,4-D ([2,4-dichlorophenoxy]acetic acid) plus MCPP (2-[4-chloro-2-methylphenoxy]propanoic acid) at sublethal rates controlled field bindweed (*Convolvulus arvensis* L.) more effectively than the herbicide mixture alone and as effectively as the pathogen at a 10-fold higher rate. Similarly, a sublethal dose of glyphosate (50 $\mu mol\ L^{-1}$) suppressed the biosynthesis of a phytoalexin derived from the shikimate pathway in sicklepod (*Cassia obtusifolia* L.) infected by *Alternaria cassiae* Jurair & Khan, reducing the resistance of the weed to fungal infection and disease development (Sharon *et al*. 1992). Although various interactions have been documented in the reports cited above, in some cases, these interactions were not mathematically classified as antagonistic, synergistic or additive effects, as extensively outlined in a review of the interactions of many types of agrochemicals (Hatzios & Penner 1985). Strategies based on the synergistic interactions of glyphosate and plant pathogens could result in improved, more effective management of these weeds and warrants further investigation.

The fungus, *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar: Fr. (IMI 364368), originally isolated from sicklepod (*Senna obtusifolia* L.), exhibited excellent biocontrol potential for several weed species, such as 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, Charlotte, NC, USA) (Walker & Tilley 1997). This fungus also effectively controlled kudzu (*Pueraria montana* var. *lobata* L.) by ⩾80% without a dew treatment over a wide range of physical and environmental conditions and under field conditions (Boyette *et al*. 2001, 2002; Hoagland *et al*. 2007). Although redvine and trumpetcreeper were not included in *M. verrucaria* host range tests, encompassing many plant species, preliminary experiments (Boyette *et al*. 2006) revealed that both redvine and trumpetcreeper were susceptible to infection by this fungus, but not at levels that would provide adequate weed control. The objectives of this present study were to determine the effects of combined and sequential applications of a glyphosate product and *M. verrucaria* on the biological control of redvine and trumpetcreeper under field conditions and to determine the effects on soybean planted in the treated areas. We also wanted to classify the possible combined effects of glyphosate and this bioherbicide as additive, antagonistic or synergistic interactions.

MATERIALS AND METHODS

Inoculum production and bioherbicide formulation

The inoculum (conidia) of *M. verrucaria* was produced in Petri dishes containing potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA). The 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 lit incubators. The resulting conidia were rinsed from the plates with sterile distilled water. The spore counts and concentrations were estimated with hemacytometers (Improved Neubauer model; AO Scientific, Buffalo, NY, USA) and the desired spore densities were prepared by adding distilled water.

Field experiments

The field test plots were established in June 2000 and 2001 in a field that was heavily infested with naturally occurring redvine and trumpetcreeper populations near Stoneville, MS, USA. The treatments consisted of: (i) glyphosate (GLY) (Touchdown; Syntenta, Greensboro, NC, USA) at 1.12 kg acid equivalent ha^{-1}; (ii) GLY + 0.2% (v/v) Silwet L-77 surfactant (SW; UAP-Loveland Products, Greeley, CO, USA); (iii) *M .verrucaria* (inoculum density of 2×10^7 conidia mL^{-1} at a volume of 900 L ha^{-1} [run-off]) (MV) + SW; (iv) MV + SW + GLY; (v) MV + SW followed by (fb) GLY 2 h after treatment (HAT); (vi) GLY fb MV + SW 2 HAT; (vii) SW; (viii) untreated (UNT); and (ix) hand-tilled. The test plots were 2 m × 2 m. To ensure the uniformity of the weed populations, the test plots were established only in areas that contained at least 50 plants of each species. Immediately prior to the treatment, the plants in the treated plots were mowed to a height of ~15 cm. Mowing ensured uniform plant height, as well as providing plant tissue wounding or injury sites that could facilitate bioherbicidal entry and disease initiation. All the applications were applied at 900 L ha^{-1} (run-off) using a pressurized backpack sprayer (Gilmour, Somerset, PA, USA).

The disease development and weed mortality data were recorded at 3 day intervals over a period of 12 days. Ten plants of each species were randomly selected and tagged prior to the treatment for disease monitoring. The extent of disease progression was based on symptom expression from 0 to 1.0, with 0 being unaffected and 1.0 being plant mortality. The symptomatology was considered "severe" at ratings of 0.80–1.0. Following the applications, soybeans (DP 5915RR; Delta Pine & Land, Scott, MS, USA) were planted in the treated areas 12 days after treatment (DAT) and, after 12 more days, the soybean heights were measured and compared to the soybean plant heights in the tilled test plots. As irrigation was not available, all the plots in 2000 were hand-watered immediately following the soybean planting in order to have sufficient moisture for soybean germination and stand establishment. As watering occurred after the applications and the ratings of the bioherbicide and/or herbicide, no interaction or interference of the bioherbicidal or herbicidal action occurred. Adequate soil moisture was available in 2001, precluding the need for additional water. The data over the 2 years were averaged, followed by subjection to Bartlett's test for homogeneity of variance (Steele *et al.* 1997). A completely randomized, block experimental design was utilized. The untransformed data are presented because the arcsine square-root transformation of the data did not alter the interpretation of the data. The means were subjected to analysis of variance and then compared with Fisher's Least Significant Difference test ($P = 0.05$) when the *F*-test from the analysis indicated significance. In the disease kinetic studies, the data were analyzed using standard mean errors and best-fit regression analysis.

Quantification of the interactions

The interactions between the herbicides in mixtures were analyzed according to Colby (1967), using the formula, $E = X_A Y_B / 100$, in which X_A and Y_B represent weed control as a percentage of the control, with herbicide A (GLY) used at dosage p and herbicide B (MV) used at dosage q, respectively. E is the expected survival as a percentage of the control for mixture A and B at dosages p and q. The observed response is obtained experimentally by comparing the activity of single compounds with mixtures containing the same rate of the constituents as applied singly. A deviation from the expected response, as calculated from the level of interaction R between the expected and the observed response of the two compounds, would indicate synergism or antagonism. By definition, additive interactions are present if $R = 1$, synergism occurs if $R > 1$, and antagonism occurs if $R < 1$. Due to the inherent biological variability of the test systems, synergism is considered significant if $R = 1.5$ and antagonism is considered significant if $R = 0.5$. Additive interactions are present when R is between 0.5 and 1.5 (Gisi *et al.* 1985).

RESULTS AND DISCUSSION

Redvine and trumpetcreeper were controlled at 12 DAT by 88% and 90%, respectively, when the fungal spores were tank-mixed with GLY (Table 1). Neither GLY alone, nor MV alone, effectively controlled either weed species, with GLY alone controlling only 40 and 45% of redvine and trumpetcreeper, respectively, and MV controlling 25 and 30% of redvine and trumpetcreeper, respectively. No significant difference occurred between any treatments containing GLY and MV, regardless of the application sequence (Table 1). The dry weight reductions of the plants followed a similar trend (Table 1). As a result of the expected survival and observed survival ratios in all of the treatments tested being >1.5 (Gisi *et al.* 1985), it is concluded that these interactions were synergistic (Table 2). In the disease kinetic studies, only the data from the plots receiving the MV + SW and GLY + MV treatments are presented because there were no significant differences in the most effective treatment effects. When treated with MV only, a linear regression provided the best fit for both weed species (Fig. 1), with R^2-values of 0.97. When treated with MV + GLY, a second degree, polynomial regression curve provided the best fit for both weed species (Fig. 2), with R^2-values of 0.99. The soybean planted in the treated plots emerged normally, with no reductions occurring in plant height or biomass (Table 3). No visual disease or herbicide damage occurred to the soybean (data not shown).

Research elsewhere has revealed that several GLY-formulated products suppressed or abolished conidial germination of the bioherbicidal fungus, *Microsphaeropsis amaranthi* (Smith & Hallett 2006). However, by testing the adjuvants commonly used in GLY products and technical-grade GLY salts, it was revealed that this inhibition was related to formulation additives and not the active ingredient. When *M. amaranthi* was applied 1–3 days after GLY, the GLY rate required to control common waterhemp (*Amaranthus rudis* Sauer) was reduced by half (Smith & Hallett 2006).

Table 1. Biological control of *Brunnichia ovata* and *Campsis radicans* by an interaction of the mycoherbicide, *Myrothecium verrucaria*, with glyphosate

Treatment†‡§¶	Weed control (%)		Dry weight reduction (%)	
	Brunnichia ovata	*Campsis radicans*	*Brunnichia ovata*	*Campsis radicans*
GLY	40b††	45b	63b	67b
GLY + SW	42b	45b	65b	65b
MV + SW	25c	30c	40c	45c
MV + SW + GLY	88a	90a	92a	92a
MV + SW fb GLY	88a	88a	87a	90a
GLY fb MV + SW	85a	85a	88a	88a
SW	0d	0d	0d	0d
UNT	0d	0d	0d	0d
Tilled	0d	0d	0d	0d

† Glyphosate rates were 1.12 kg acid equivalent ha^{-1}; ‡ the data were collected at 12 days after treatment. For those plots receiving two treatments, the second application was made 2 h after the initial application; § the inoculum rate was 2×10^7 conidia mL^{-1}; ¶ Silwet L-77 was added to make a 0.2% solution; †† the means followed by the same letter do not differ according to the $FLSD_{05}$; fb, followed by; GLY, glyphosate; MV, *Myrothecium verrucaria*; SW, Silwet L-77 surfactant; UNT, untreated.

Table 2. Interaction between *Myrothecium verrucaria* and glyphosate for controlling *Brunnichia ovata* and *Campsis radicans*

Treatment	Observed survival (%)		Expected survival (%)†		R‡	
	Brunnichia ovata	*Campsis radicans*	*Brunnichia ovata*	*Campsis radicans*	*Brunnichia ovata*	*Campsis radicans*
GLY	60	55	60	55	1.00	1.00
MV + SW	75	70	75	70	1.00	1.00
MV + SW + GLY	12	10	45	39	3.75	3.90
MV + SW fb GLY	12	12	45	39	3.75	3.25
GLY fb MV + SW	15	15	45	39	3.00	2.60

† Expected values were determined by the Colby equation, $E_1 = (X_1 Y_1)/100$, where E1 is the expected weed survival with components A + B, X_1 is the observed weed survival with A (glyphosate), and Y_1 is the observed mortality with B (*Myrothecium verrucaria*); ‡ the ratio between the expected and observed survival (R = expected/observed); R-values >1.5 are considered to be synergistic (Gisi *et al.* 1985). fb, followed by; GLY, glyphosate; MV, *Myrothecium verrucaria*; SW, Silwet L-77 surfactant.

In the results reported herein, there were no significant differences either in the weed control or dry weight reductions when GLY was applied with, prior to or after fungal treatments (Table 1). We have shown previously that, under controlled environmental conditions, a synergistic effect occurred when MV was applied 2 days after the application of the GLY product, Roundup-Ultra (Monsanto, St Louis, MO, USA), to redvine (Boyette *et al.* 2006). In these studies, the additive effects occurred when GLY was applied either 2 days before or 2 days after MV application and when GLY and MV were applied simultaneously as a mixture to redvine. The additive effects also occurred when GLY was applied either 2 days before or 2 days after MV application to trumpetcreeper. An antagonistic interaction was noted when GLY and MV were applied simultaneously to trumpetcreeper (Boyette *et al.* 2006). Recently, research in our laboratory has shown different proprietary formulations of GLY, as well as other commonly used chemical pesticides, have dramatically varying effects on conidial germination, radial growth, and the biocontrol efficacy of MV (Weaver *et al.* 2006). It is possible that the variations in GLY formulations (e.g. surfactants) might have interacted negatively with the bioherbicide. As new sprouts can arise from the underground root stock of these weeds (Chachalis *et al.* 2001), it is possible that two applications (fall and spring) might prevent the regrowth of these weeds. The application timing and other factors

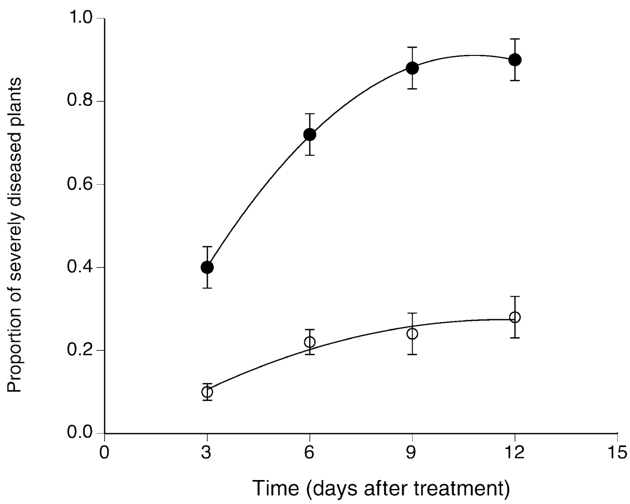

Fig. 1. Disease progression of *Myrothecium verrucaria* infecting *Brunnichia ovata*. For the plants inoculated with *M. verrucaria* only, the relationship is best described by the equation: $Y = -0.048 + 0.176X - 0.023X^2$; $R^2 = 0.96$. For the plants inoculated with glyphosate and with *M. verrucaria*, the relationship is best described by the equation: $Y = 0.028 + 0.497X - 0.068X^2$; $R^2 = 0.99$. The error bars represent ±1 standard error of the mean. (●), *M. verrucaria* and glyphosate; (○), *M. verrucaria* alone.

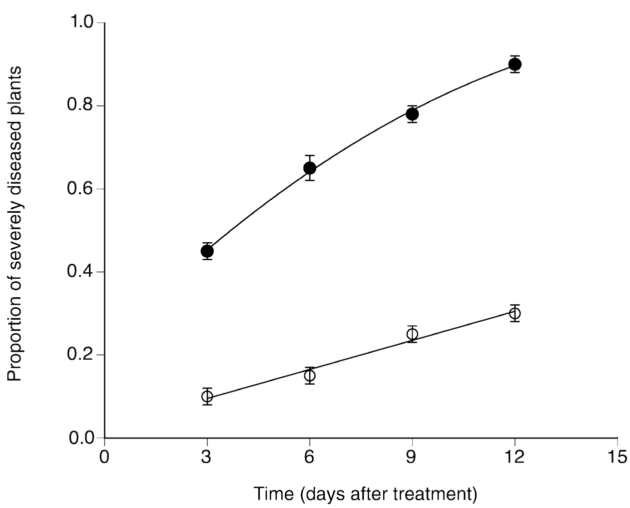

Fig. 2. Disease progression of *Myrothecium verrucaria* infecting *Campsis radicans*. For the plants inoculated with *M. verrucaria* only, the relationship is best described by the equation: $Y = 0.250 + 0.07X$; $R^2 = 0.98$. For the plants inoculated with glyphosate and with *M. verrucaria*, the relationship is best described by the equation: $Y = 0.225 + 0.248X - 0.02X^2$; $R^2 = 0.99$. The error bars represent ±1 standard error of the mean. (●), *M. verrucaria* and glyphosate; (○), *M. verrucaria* alone.

Table 3. Effects of *Myrothecium verrucaria*, adjuvants, and glyphosate interaction on soybean growth and biomass

Treatment	Soybean height reduction (%)	Biomass reduction (%)
GLY	37a†	25a
GLY + SW	30b	25a
MV + SW	35ab	23a
MV + SW + GLY	15c	10b
MV + SW fb GLY	12c	8b
GLY fb MV + SW	10c	5b
SW	35ab	25a
UNT	37a	30a
Tilled	0d	0c

† Means followed by the same letter do not differ according to the $FLSD_{05}$. fb, followed by; GLY, glyphosate; MV, *Myrothecium verrucaria*; SW, Silwet L-77 surfactant; UNT, untreated.

affecting herbicide uptake, translocation, and the control of redvine have been studied (Shaw & Mack 1991; Reddy 2000, 2005). Research is underway to examine some of these parameters with respect to combinations of GLY and MV for redvine and trumpetcreeper control.

We conclude that there was a synergistic effect on the control of redvine and trumpetcreeper when MV and the GLY product, Touchdown, were tank-mixed or applied sequentially. As the weed control was not increased by an additional surfactant (SW), it was concluded that the increased weed control was related to the synergy between MV and this formulation of GLY. Neither the weed control nor dry weight reductions of either weed species was affected by the timing of the applications. The soybean that was planted at 12 DAT in the treated plots was not adversely affected by any of the treatments. Whether these effects are related to interactions with pathogen spore germination and growth or to effects on weed defenses, as suggested in other weed: pathogen systems (Smith & Hallett 2006), will be a subject of future research in our laboratory.

These results suggest that it is possible to enhance the bioherbicidal potential of MV through synergistic interactions with chemical herbicides, such as GLY. The bioherbicidal potential of this particular strain of MV has been thoroughly established, based on findings both in our laboratory and elsewhere (Walker & Tilley 1997; Boyette *et al.* 2002, 2006; Anderson & Hallett 2004). It has been shown that many isolates of MV, including the isolate used in these studies, produce a variety of mycotoxins, including the verrucarin and roridin groups of the macrocyclic trichothecenes (Abbas *et al.* 2001, 2002). As these trichothecenes are extremely potent mycotoxins, they should be considered potentially hazardous (Mortimer *et al.* 1971; Jarvis *et al.* 1985; Abbas *et al.* 2001, 2002). Our future research also will focus on developing cultural, chemical, genetic, or combinations of these techniques, to mitigate the mycotoxins to acceptable levels of risk.

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