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Interaction of a *Myrothecium verrucaria* Mycelial Preparation and a Glyphosate Product for Controlling Redvine (*Brunnichia ovata*) and Trumpet Creeper (*Campsis radicans*)

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Abstract

A mycelial formulation of the bioherbicidal fungus *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar: Fr. (MV) was tested alone and in combination with a commercially available glyphosate [*N*-(phosphonomethyl)glycine] (GLY) product for controlling the invasive vines, redvine [*Brunnichia ovata* (Walt.) Shinners], and trumpet creeper [*Campsis radicans* (L.) Seem. ex Bureau] in field experiments conducted near Stoneville, MS. Several application timing regimens were evaluated (Fall, Spring, Fall + Spring, and Spring + Fall). We found that a Fall + Spring application of MV + GLY controlled redvine and trumpet creeper by 95%, 12 days after the second treatment, through a synergistic interaction of the fungus and glyphosate. Disease symptomatology was characterized by rapid necrosis of leaf and stem tissues, with mortality occurring within 72 h. Neither glyphosate alone, nor MV alone, effectively controlled either weed species under any application timing regimen. No visual disease or herbicide damage occurred on glyphosate-resistant soybean plants in the treated test plots. These results suggest that some formulations of glyphosate, mixed with the bioherbicide MV, can effectively control redvine and trumpet creeper, two of the most troublesome weeds in the row crops of the Mississippi Delta region in the mid-southern U.S.

Keywords

Bioherbicide, Interaction, Mycelial Formulation, *Myrothecium verrucaria*, *Campsis radicans*, *Brunnichia ovata*

1. Introduction

Redvine [*Brunnichia ovata* (Walt.) Shinners] and trumpet creeper [*Campsis radicans* (L.) Seem. ex Bureau] are native perennial, deciduous, woody, dicotolydenous, vines capable of growing several meters in length [1]. These weeds are distributed extensively in the lower Mississippi Alluvial Plain area (Mississippi Delta) of the southern U.S., often found in dense populations in cultivated and fallowed fields, wastelands, fence rows, yards, riverbanks, swamps, and forests. These weeds have been rated among the ten most troublesome weeds in the row crops of the Mississippi Delta region, since they reduce crop yield and quality, and interfere with cultivation and harvest operations [1].

Glyphosate [*N*-(phosphonomethyl)glycine] has become the predominant post-emergence herbicide used in soybean [*Glycine max* (L.) Merr.] in the U.S., with approximately 90% of soybean acres sown to glyphosate-resistant varieties [2]. Glyphosate is a broad-spectrum post-emergence herbicide with some herbicidal activity on redvine and trumpet creeper [3] [4] [5]. However, control of these weeds with glyphosate alone, even at rates two-to-four times the rates recommended in non-GMO soybeans, is temporary at best and when used alone cannot satisfactorily control these weeds [5].

The use of fungi and bacteria as inundative biological control agents (bioherbicides) has been recognized as both a viable method for controlling agronomic weed pests and a significant technological alternative to chemical herbicides [6]-[11]. Considerable interest exists worldwide regarding bioherbicides [9] [10] [11] [12] and there is an acute need to develop non-chemical or bioherbicidal weed management tools and strategies as alternatives to chemical weed control as summarized in several reviews [8]-[18].

The fungus *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar: Fr., (IMI 368023; MV) originally isolated from sicklepod (*Senna obtusifolia* (L.) Irwin & Barneby) 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 [SW; a silicone-polyether copolymer spray adjuvant, OSi Specialties, Inc., Charlotte, NC, 28701] [19] [20]. MV also effectively controlled kudzu [*Pueraria montana* var. *lobata* Willd. (Ohwii)] over a wide range of physical and environmental conditions, and under field conditions [20]-[25]. Although redvine and trumpet creeper were not included in MV host range tests encompassing many plant species [19], later experiments revealed that both redvine and trumpet creeper were susceptible to infection by spores of this fungus, but not at levels that would provide adequate weed control [26]. In subsequent research conducted under field conditions near Stoneville, MS, USA, MV spore formulations applied alone and in combination with a glyphosate product (Touchdown™) controlled natural infestations of redvine and trumpet creeper 88% and 90%, respectively, through a synergistic interaction of the fungus and glyphosate [27]. Neither glyphosate alone, nor MV alone, controlled these weeds at commercially acceptable levels (80%).

Although MV can potentially control several weed species, its production of macrocyclic trichothecenes (mycotoxins) by fungal spores [28] [29] [30] [31] is an issue that limits its practical usage. Thus, a biologically effective, mycotoxin-free formulation could greatly expand the bioherbicidal potential of this bioherbicide. Experiments in our laboratory had previously demonstrated that a spore-free liquid culture of MV, comprised of mycelia fragments (void of trichothecenes), exhibited high bioherbicidal activity against the weed kudzu under field conditions [32]. Due to the high efficacy exhibited by MV mycelia against several weeds [20] [33] [34] [35] [36], we hypothesized that a mycelial formulation of MV, used in conjunction with a compatible glyphosate product, may also have potential for controlling redvine and trumpet creeper. Thus, the objectives of this present study were to determine the effects of combined applications of a glyphosate product (Touchdown™) and a mycelial-based MV formulation on biological control of redvine and trumpet creeper in field plots where greenhouse-grown seedings of these weeds had been transplanted, and to characterize possible combined effects of glyphosate and this bioherbicide as additive, antagonistic, or synergistic interactions.

2. Materials and Methods

2.1. MV Source and Production

MV cultures [*M. verrucaria* (IMI 368023)] were grown and maintained in petri dishes on potato dextrose agar (PDA) (Difco Laboratories, Inc., Detroit, MI, USA) at 25˚C. Mycelial cultures of MV used in these experiments were prepared as described previously [32]. Briefly, a fermenter (Model MF-214, New Brunswick Corp., Edison, NJ, USA) containing a soy flour-corn meal medium (15.0 g soybean flour, 3.75 g corn flour, 30.0 g sucrose and 3.0 g calcium carbonate per L distilled H_2O) was inoculated under sterile conditions with starter inoculum (mycelial preparation grown in shake-flasks). The shake-flask medium (soy flour-corn meal) was inoculated with a 10 mm agar plug (~10^6 spores·mL^{-1}) from a petri dish of MV spores. The flask was incubated on a rotary shaker (185 - 200 rpm, 28˚C, 7 days) and mycelial fungal growth proceeded without spore production. The MV mycelial product produced via fermentation for 48 - 72 h was harvested and stored at 4˚C until use. Concentrations of the mycelial formulations used in these tests were based on percent (v/v basis) of the fermentation batch as described elsewhere [36]. That procedure consisted of determining the viable propagule density (colony forming units; cfu) of the MV mycelial fermentation product in diluted samples (1.0 mL product:1.0 L sterile H_2O) after thoroughly mixing under sterile conditions, then plating aliquots of the mixture onto PDA in petri dishes, incubation of plates (28˚C), and lastly counting colonies after 48 h growth. Dry weight analysis of freshly prepared MV mycelium revealed that ca 50 g·L^{-1} were produced. Appropriate dilutions were made to obtain a concentration of 2.0×10^7 cfu·mL^{-1} for further studies.

The MV mycelial product used in these studies was analyzed for trichothe-

cenes as described [32]. Samples of raw culture fermentation product (mycelium and unspent medium) were extracted by shaking in ethanol [1:1 culture product:ethanol (v:v)] at 22˚C for ≥ 2 h at 125 rpm. The mixture was centrifuged (14,000 RCF, 10 min, to remove particulate matter, and 1.0 mL of the clarified supernatant was transferred to amber glass vials. Trichothocenes were detected by UV absorbance after separation on a Polar Advantage II monolithic column (3 μm, 4.6 × 50 mm, Dionex, Sunnyvale, CA, USA) using a Dionex HPLC system. The mobile phase was: (A) 0.1% acetic acid in water and (B) 0.1% acetic acid in acetonitrile. Binary gradient elution starting conditions were: 35% B for 2 min, followed by an increase to 50% B at 4.5 min; 58% at 6 min; 70% at 7.29 min; 80% at 7.84 min; 95% at 8.5 min, holding for 0.5 min before returning to initial conditions. Roridin A and verrucarin A (Sigma Chemical Co., St. Louis, MO, USA) were used as standards. The limit of detection in this protocol was 2 $\mu g \cdot mL^{-1}$ [32].

2.2. Greenhouse

Seed Sources and Greenhouse Planting

Trumpet creeper and redvine seeds were collected from local sites near Stoneville, MS and stored at 0˚C - 4˚C. Seeds were planted in a 1:1 mixture of commercial potting mix (Jiffy Products of America, Inc., Batavia, IL, USA) and Bosket sandy loam contained in plastic trays (25 × 52 cm) and placed on greenhouse benches to foster germination, emergence and early growth. The soil mixture was supplemented with a controlled-release (14:14:14, N:P:K) fertilizer (Grace Sierra Horticultural Products, Milpitas, CA, USA). Greenhouse temperatures ranged from 28˚C to 32˚C with 40% to 60% relative humidity. The photoperiod was approximately 14 h, with 1600 to 1800 $\mu E \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation at midday. Water was routinely supplied. Plants were transplanted to 2-inch square peat-pots (one plant per pot) at the 1 to 2 leaf growth stage and grown to the 3 to 4 leaf growth stage.

2.3. Transplantation of Seedlings to Establish Field Plots

Field test plots were established in early summer (June) in a field near Stoneville, MS Coordinates: (Coordinates: 33˚25'28"N 90˚52'53"W) that was void of redvine and trumpet creeper. Plants grown under greenhouse conditions (as described above) were transplanted at the 3 to 4 leaf growth stage into field plots. At the time of transplanting, holes (10 - 15 cm diam. × 10 - 15 cm deep) were hand-dug with a hoe, water (ca. 1 L) was added, one peat pot containing a single redvine or trumpet creeper plant was placed in the hole, soil was hipped-up around each plant, followed by additional watering of soil on the surface (ca. 2 L). Plants were watered every three days, or as needed. Plots were 4.0 m × 4.0 m, consisting of 8 rows on 50 cm centers, with each row containing 14 plants of either redvine or trumpet creeper plants (28 cm apart) in alternating sequences. The plants were allowed to establish healthy root systems for ca. 16 months before treatments

were made. Dead or non-uniform plants were replaced with healthy greenhouse-grown plants.

2.4. Bioherbicide and Herbicide Treatments of Field Plants

Four application timing regimens were utilized: 1) Fall, (1 application, October); 2) Spring (1 application, May); 3) Fall + Spring (2 applications, October and May); and 4) Spring + Fall (2 applications, May and October). Treatments consisted of: 1) MV (mycelium inoculum density of 1.0×10^7 cfu·mL^{-1} at a volume of 100 L·ha^{-1}) + Silwet L-77 (0.20%) surfactant (SW); 2) glyphosate [GLY (Touchdown^{TM}) + SW]; 3) MV + GLY + SW; 4) SW only; 5) untreated (UNT). Immediately prior to treatment, plants were mowed to uniform height (~15 cm). Glyphosate was applied at a rate of 1.12 kg a.i. ha^{-1}. All applications were applied at 100 L·ha^{-1} using a pressurized backpack sprayer. Twenty-five plants of each species were randomly selected and tagged prior to treatment for assessment of plant injury and weed control days after treatment (DAT). The extent of plant injury was based on symptom expression from 0 to 1.0, where 0 represented unaffected plant tissues and 1.0 represented plant mortality. The selected plants were monitored over 12 days for injury development. Plant injury was considered "severe", and weeds were considered "controlled" at ratings of 0.80 to 1.0. After this data was collected, the above-ground plant material from the selected plots was harvested and dried at 80˚C for 72 h and biomass values were recorded for each sample. For treatments receiving two treatments, mortality and dry weight determinations were conducted 12 days after the second treatment. Following the Spring treatment applications, soybeans (DP 5915RR) were planted in the treated areas 40 DAT, soybean plant heights were measured and compared to soybean plant heights in the untreated plots. A randomized complete block experimental design with four replications was utilized. The mean percentages of plant mortalities and biomass reductions were calculated for each treatment and subjected to Arcsin transformation. The transformed data were statistically compared using analysis of variance (ANOVA) ($P = 0.05$). Results were back-transformed to the original measurements (percentages) for presentation. Data were analyzed via the PROC MIXED function of SAS v9.3 (SAS Institute, Cary, NC, USA) using the least significant difference of 0.05. In the plant injury kinetic studies, data were analyzed using standard mean errors and best-fit regression analysis. The experiment was repeated, and data over the two years were averaged, followed by subjection to Bartlett's test for homogeneity of variance [37].

2.5. Quantification of Interactions

Interactions between components in mixtures were analyzed according to Colby [38], 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 (glyphosate) used at dosage p and (bio)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, could indicate synergism or antagonism. By definition, interactions are classified as additive when R = 1.0, synergistic when R > 1.0 and antagonistic when R < 1.0. Due to the inherent biological variability of the test systems, synergistic and antagonistic interactions are considered statistically significant when R ≥ 1.5 and R ≤ 0.5, respectively and additive interactions are significant at R values between 0.5 and 1.5 [39].

3. Results and Discussion

Redvine and trumpet creeper were each controlled 95% in the Fall + Spring timing regimen 12 DAT when MV mycelia preparations were tank-mixed with glyphosate (**Figure 1(a)** & **Figure 2(a)**). The Fall + Spring application regimen provided significantly greater control of trumpet creeper (95%) than that from the other timing regimens (85% for Fall, Spring, or Spring + Fall). However, a single Fall treatment of MV + GLY + SW controlled redvine at levels that were not statistically different than levels achieved by a Fall + Spring treatment (90% and 95%, respectively) (**Figure 1(a)**). Neither glyphosate + SW alone, nor MV + SW alone, effectively controlled either weed under any of the timing regimens (**Figure 1(a)** and **Figure 2(a)**). Dry weight reductions of plants followed a similar trend (**Figure 1(b)** & **Figure 2(b)**). Because the expected survival and observed survival ratios of both redvine and trumpet creeper receiving an application of MV + GLY + SW were greater than 1.5 [39], it is concluded that these interactions were synergistic (**Table 1**). In the plant injury, kinetic studies, only data from plots receiving the MV + SW, MV + GLY, and MV + GLY + SW treatments are presented. In the Fall application regimen, redvine plants treated with MV + SW, GLY + SW, or MV + GLY + SW, a 2nd degree polynomial regression curve provided the best fit for each curve (R^2 = 0.96, 0.98, and 0.96, respectively) (**Figure 3(a)**). Similarly, 2nd degree polynomial regression curves also provided the best fit for each curve (R^2 = 0.96, 0.98, and 0.98, respectively) for those treatments applied to trumpet creeper in the Fall regimen (**Figure 3(a)**). In the Spring regimen, redvine plants treated with MV + SW, GLY + SW, or MV + GLY + SW, a 2nd degree polynomial regression curve provided the best fit for each curve (R^2 = 0.96, 0.98, and 0.96, respectively) (**Figure 3(b)**). When trumpet creeper plants were treated with MV + SW, GLY + SW, or MV + SW + GLY in the Spring regimen, 2nd degree polynomial regression curves also provided the best fit for each curve (R^2 = 0.98, 0.96, and 0.98, respectively) (**Figure 3(b)**). Soybeans planted in treated plots emerged normally and no reductions in plant height occurred (data not shown).

We have previously shown that, under controlled environmental conditions, a synergistic effect occurred when MV spores were applied two days after

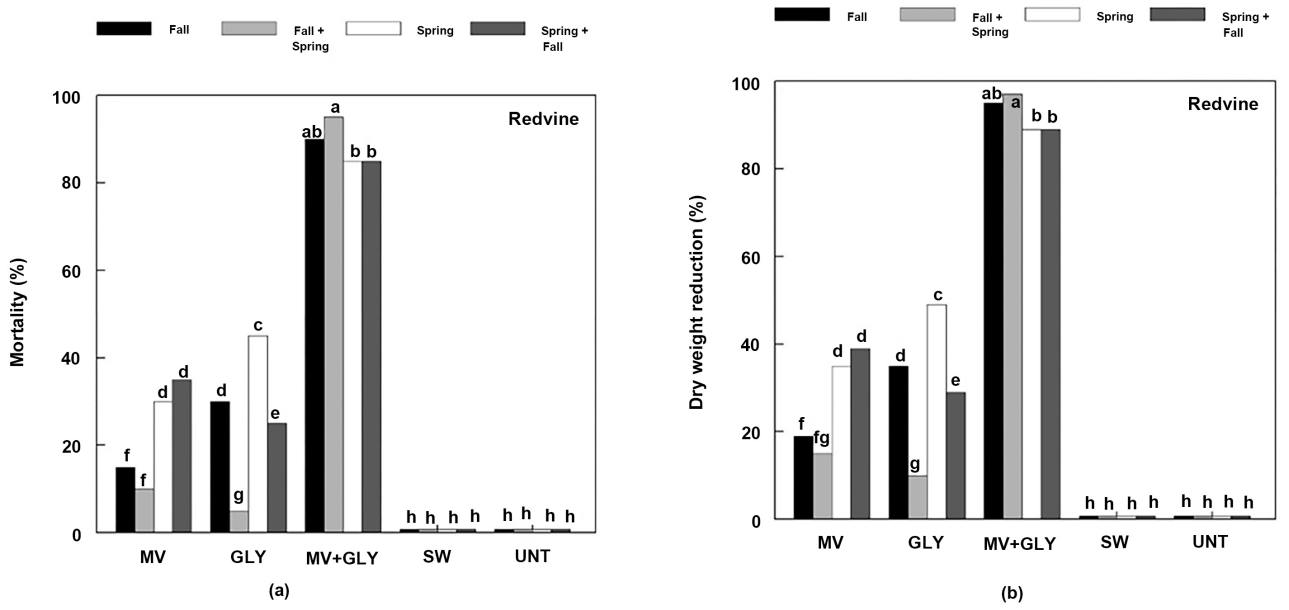

Figure 1. Effects of spray applications of MV, glyphosate, and combinations of MV and glyphosate on redvine seedling upon (a) plant mortality and (b) dry weight reduction 12 DAT. For treatments receiving two treatments, mortality and dry weight determinations were conducted 12 DAT (second treatment). Histogram bar values with the same letter are not significantly different at $p = 0.05$, according to Fisher's Least Significant Difference.

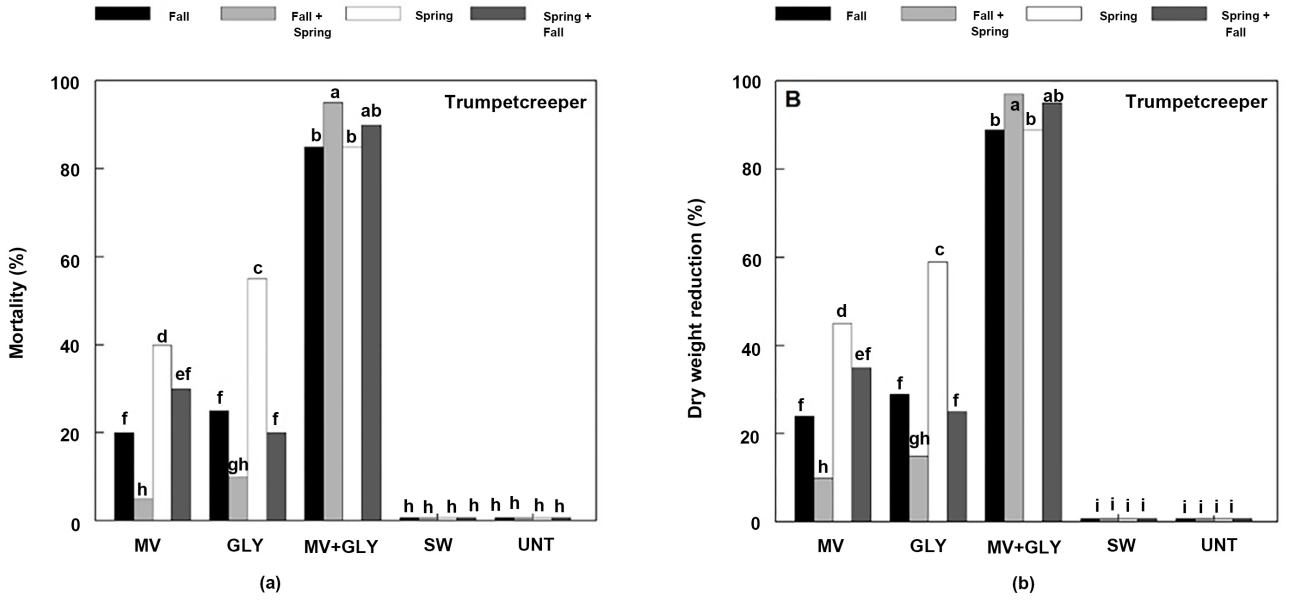

Figure 2. Effects of spray applications of MV, glyphosate, and combinations of MV and glyphosate on trumpet creeper seedling upon (a) plant mortality and (b) dry weight reduction 12 days after treatment. For treatments receiving two treatments, mortality and dry weight determinations were conducted 12 DAT (second treatment). Histogram bar values with the same letter are not significantly different at $p = 0.05$, according to Fisher's Least Significant Difference.

application of the glyphosate product Roundup-Ultra™ (Monsanto Corp., St. Louis, MO 63167) to redvine and trumpet creeper, while an antagonistic interaction occurred when glyphosate and MV spores were applied simultaneously to plants [26]. Subsequent field experiments revealed that MV spore formulations

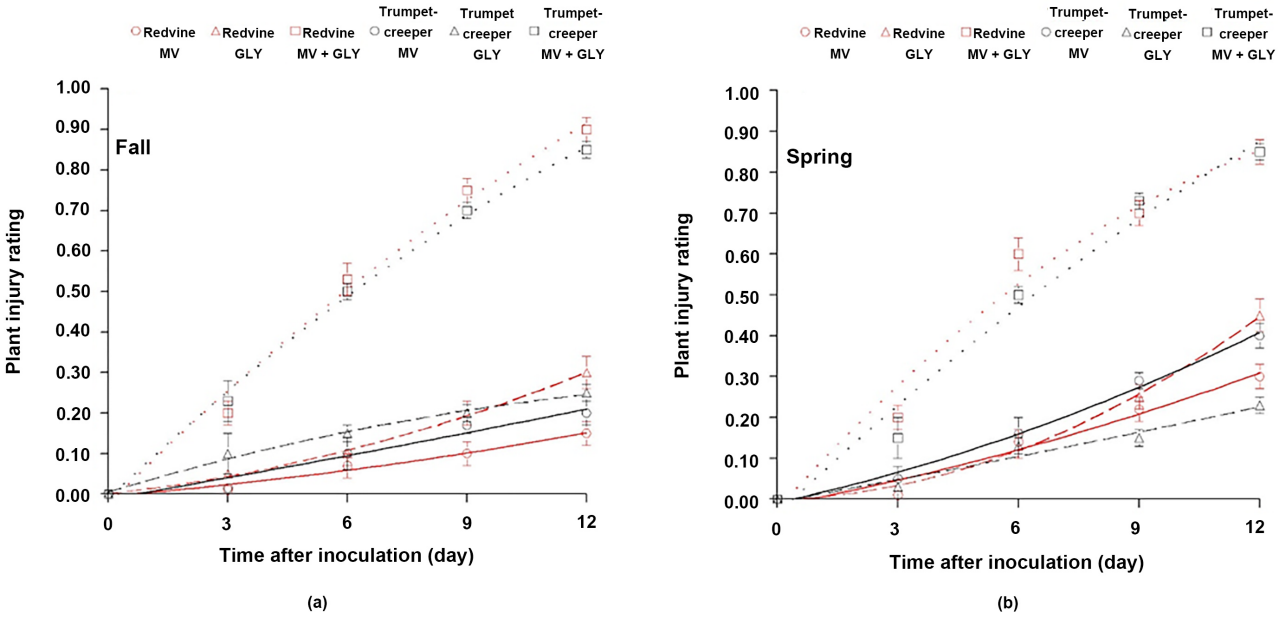

Figure 3. Plant injury progression to redvine (red markers) and trumpet creeper (black markers) during: (a) Fall or (b) Spring timing regimens. Open circles = MV + GLY; Open triangles = GLY only; and open squares = MV + GLY + SW. For redvine plants inoculated in the Fall application regimen, the relationship for MV + SW is best described by the equation: $Y = -0.0049 + 0.0082X + 0.0004X^2$, $R^2 = 0.99$; for GLY + SW, the relationship is best described by the equation, $Y = 0.0014 + 0.0107X + 0.0012X^2$, $R^2 = 0.99$; for MV + GLY + SW, the relationship is best described by the equation, $Y = -0.024 + 0.098X - 0.0017X^2$, $R^2 = 0.99$. For trumpet creeper plants inoculated in the Fall application regimen: the relationship for MV + SW is best described by the equation, $Y = -0.0119 + 0.0171X + 0.0001X^2$, $R^2 = 0.96$; for GLY + SW, the relationship is best described by the equation, $Y = 0.0057 + 0.0295X - 0.0008X^2$, $R^2 = 0.98$; for MV + GLY + SW, the relationship is best described by the equation, $Y = -0.0109 + 0.0942X - 0.0018X^2$, $R^2 = 0.98$. For redvine plants inoculated in the Spring application regimen, the relationship for MV + SW is best described by the equation: $Y = -0.0151 + 0.0184X + 0.0007X^2$, $R^2 = 0.99$; for GLY + SW, the relationship is best described by the equation, $Y = -0.0006 + 0.0030X + 0.0029X^2$, $R^2 = 0.99$; for MV + GLY + SW, the relationship is best described by the equation, $Y = -0.0271 + 0.1114X - 0.0032X^2$, $R^2 = 0.99$. For trumpet creeper plants inoculated in the Spring application regimen, the relationship for MV + SW is best described by the equation: $Y = -0.008 + 0.0213X + 0.0011X^2$, $R^2 = 0.99$; for GLY + SW, the relationship is best described by the equation, $Y = -0.0051 + 0.0174X + 0.0002X^2$, $R^2 = 0.98$; for MV + GLY + SW, the relationship is best described by the equation, $Y = -0.0357 + 0.09931X - 0.0014X^2$, $R^2 = 0.96$. Error bars = ±1 SEM.

Table 1. Interaction of *Myrothecium verrucaria* and glyphosate relative to plant survival of *Brunnicia ovata* (redvine) and *Campsis radicans* (trumpet creeper) as analyzed using Colby's method [38].

	Fall			Fall/Spring			Spring			Spring/Fall		
Treatment	Survival (%)			Survival (%)			Survival (%)			Survival (%)		
	Observed[a]	Expected[b]	R[c]	Observed[a]	Expected[b]	R[c]	Observed[a]	Expected[b]	R[c]	Observed[a]	Expected[b]	R[c]
Redvine												
MV	85	85	1	90	90	1	70	70	1	65	65	1
GLY	70	70	1	95	95	1	55	45	1	75	75	1
MV + GLY	10	60	6	5	86	17	15	39	2.6	15	49	3
Trumpet creeper												
MV	80	80	1	95	95	1	60	60	1	70	70	1
GLY	75	75	1	90	90	1	45	45	1	80	80	1
MV + GLY	15	60	4	5	86	17	15	27	2	10	56	6

[a]Observed survival (percent of control) of plants treated with MV, GLY, or MV + GLY. [b]Expected values were determined using the Colby [38] equation: E = (X)(Y)/100; E is the expected plant survival (expressed as percent of control) and components X and Y represent MV and GLY, respectively. [c]R values ≥ 1.5 are considered to be synergistic [39] and additive interactions are noted when R is between 0.5 and 1.5.

applied in combination with a glyphosate product (Touchdown™) controlled natural infestations of redvine and trumpet creeper 88% and 90%, respectively, through a synergistic interaction of the fungus and glyphosate [27]. Neither glyphosate alone, nor MV alone, controlled these weeds at commercially acceptable levels (80%). Other plant pathogens have also been shown to exhibit synergistic interactions with glyphosate [40] [41] [42] [43] and application of MV plus glyphosate resulted in a synergistic interaction for hemp sesbania control [44].

Other research in our laboratory has shown that different proprietary glyphosate formulations, as well as other commonly used chemical pesticides, have dramatically varying effects on conidial germination, radial growth, and the biocontrol efficacy of MV, suggesting that variations in glyphosate formulations (e.g., surfactants) may have interacted negatively with the bioherbicide [45].

In these present studies, we conclude that there was a synergistic effect upon weed control of redvine and trumpet creeper when a MV mycelial product and the glyphosate product Touchdown™ were tank-mixed. Because weed control was not increased by additional surfactant (SW), it was concluded that the increased weed control was due to synergy between MV mycelia and glyphosate. These findings are corroborated by other research using high purity glyphosate (without adjuvants/surfactants). In those studies, technical-grade glyphosate was not toxic to MV growth and sporulation at concentrations up to 2.0 mM when grown on agar supplemented with the herbicide, and an interaction of glyphosate plus MV mycelia was synergistic in controlling Palmer amaranth (*Amaranthus palmeri* S. Wats.) [36]. The bioherbicidal potential of this strain of MV has been thoroughly established, based on findings both in our laboratory and elsewhere [19] [20] [22] [26] [33] [34] [35] [36]. The results reported herein suggest that it is possible to enhance the bioherbicidal potential of a mycelial formulation of MV through synergistic interactions with compatible chemical herbicides, such as glyphosate and to control aggressive and established weeds such as redvine and trumpet creeper under field conditions.

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Note

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.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Elmore, C.D. (1984) Perennial Vines in the Delta of Mississippi. In: *Mississippi Agricultural & Forestry Experiment Station Bulletin* 927, Mississippi State University, Mississippi State, 9.

[2] Duke, S.O. (2018) The History and Current Status of Glyphosate. *Pest Management Science*, **74**, 1027-1034. https://doi.org/10.1002/ps.4652

[3] Chachalis, D. and Reddy, K.N. (2000) Factors Affecting *Campsis radicans* Seed Germination and Seedling Emergence. *Weed Science*, **48**, 212-216. https://doi.org/10.1614/0043-1745(2000)048[0212:FACRSG]2.0.CO;2

[4] Chachalis, D., Reddy, K.N. and Elmore, C.D. (2001) Characterization of Leaf Surface, Wax Composition, and Control of Redvine and Trumpet Creeper with Glyphosate. *Weed Science*, **49**, 156-163. https://doi.org/10.1614/0043-1745(2001)049[0156:COLSWC]2.0.CO;2

[5] Reddy, K.N. and Chachalis, D. (2004) Redvine (*Brunnichia ovata*) and Trumpet creeper (*Campsis radicans*) Management in Glufosinate- and Glyphosate-Resistant Soybean. *Weed Technology*, **18**, 1058-1064. https://doi.org/10.1614/WT-03-234R1

[6] Hoagland, R.E. (1990) Microbes and Microbial Products as Herbicides. American Chemical Society Symposium Series No. 439. ACS Books, Washington DC. https://doi.org/10.1021/bk-1990-0439

[7] TeBeest, D.O. (1991) Microbial Control of Weeds. Chapman and Hall, New York. https://doi.org/10.1007/978-1-4615-9680-6

[8] Rosskopf, E. (1999) Use of Plant Pathogens in Weed Control. In: *Handbook of Weed Control*, Academic Press, New York, 891-911. https://doi.org/10.1016/B978-012257305-7/50082-5

[9] Charudattan, R. (2001) Biological Control of Weeds by Means of Plant Pathogens: Significance for Integrated Weed Management in Modern Agro-Ecology. *BioControl*, **46**, 229-260. https://doi.org/10.1023/A:1011477531101

[10] Charudattan, R. (2005) Ecological, Practical, and Political Inputs into Selection of Weed Targets: What Makes a Good Biological Control Target? *Biological Control*, **35**, 183-196. https://doi.org/10.1016/j.biocontrol.2005.07.009

[11] Weaver, M.A., Lyn, M.E., Boyette, C.D. and Hoagland, R.E. (2007) Bioherbicides for Weed Control. In: Upadhyaya, M.K. and Blackshaw, R.E., Eds., *Non-Chemical Weed Management*, CAB International, New York, 93-110. https://doi.org/10.1079/9781845932909.0093

[12] Glare, T., Caradus, J., Gelemter, W., Jackson, T., Keyhani, N., Kohl, J., Marrone, P., Morin, L. and Stewart, A. (2012) Have Biopesticides Come of Age? *Trends in Biotechnology*, **30**, 250-258. https://doi.org/10.1016/j.tibtech.2012.01.003

[13] Boyetchko, S.M., Rosskopf, E.N., Caesar, A.J. and Charudattan, R. (2002) Biological Weed Control with Pathogens: Search for Candidates to Applications. In: Khachatourians, G.G. and Arora, D.K., Eds., *Applied Mycology and Biotechnology*, Volume 2, Elsevier, Amsterdam, 239-274. https://doi.org/10.1016/S1874-5334(02)80013-2

[14] Hoagland, R.E. (2001) Microbial Allelochemicals and Pathogens as Bioherbicidal Agents. *Weed Technology*, **15**, 835-857. https://doi.org/10.1614/0890-037X(2001)015[0835:MAAPAB]2.0.CO;2

[15] Boyetchko, S. and Peng, G. (2004) Challenges and Strategies for Development of Mycoherbicides. In: Arora, D.K., Ed., *Fungal Biotechnology in Agricultural, Food, and Environmental Applications*, Marcel Dekker, New York, 11-121.

https://doi.org/10.1201/9780203913369.ch10

[16] Hallett, S.G. (2005) Where Are the Bioherbicides? *Weed Science*, **53**, 404-415. https://doi.org/10.1614/WS-04-157R2

[17] Duke, S.O., Scheffler, B.E., Boyette, C.D. and Dayan, F.E. (2015) Biotechnology in Weed Control. In: Kirk-Othmer, Ed., *Encyclopedia of Chemical Technology*, John Wiley & Sons, Inc., New York, 1-25. https://doi.org/10.1002/0471238961.herbduke.a01.pub2

[18] Hoagland, R.E. and Boyette, C.D. (2016) Controlling Herbicide-Susceptible, -Tolerant and -Resistant Weeds with Microbial Bioherbicides. *Outlooks on Pest Management*, **27**, 256-266. https://doi.org/10.1564/v27_dec_04

[19] Walker, H.L. and Tilley, A.M. (1997) *Myrothecium verrucaria* from Sicklepod (*Senna obtusifolia*) as a Potential Mycoherbicide Agent. *Biological Control*, **10**, 104-112. https://doi.org/10.1006/bcon.1997.0559

[20] Boyette, C.D., Hoagland, R.E. and Stetina, K.C. (2014) Biological Control of the Weed Hemp Sesbania (*Sesbania exaltata*) in Rice (*Oryza sativa*) by the Fungus *Myrothecium verrucaria*. *Agronomy*, **4**, 74-89. https://doi.org/10.3390/agronomy4010074

[21] Boyette, C.D., Walker, H.L. and Abbas, H.K. (2001) Control of Kudzu with a Fungal Pathogen Derived from *Myrothecium verrucaria*. U.S. Patent No. 6,274,534.

[22] Boyette, C.D., Walker, H.L. and Abbas, H.K. (2002) Biological Control of Kudzu (*Pueraria lobata*) with an Isolate of *Myrothecium verrucaria*. *Biocontrol Science and Technology*, **11**, 75-82. https://doi.org/10.1080/09583150120093031

[23] Hoagland, R.E., Weaver, M.A. and Boyette, C.D. (2007) *Myrothecium verrucaria*: Bioherbicide, and Strategies to Reduce Its Non-Target Risks. *Allelopathy Journal*, **19**, 179-192.

[24] Weaver, M.A., Boyette, C.D. and Hoagland, R.E. (2012) Bioherbicidal Activity from Washed Spores of *Myrothecium verrucaria*. *World Journal of Microbiology and Biotechnology*, **28**, 1941-1946. https://doi.org/10.1007/s11274-011-0996-8

[25] Weaver, M.A., Boyette, C.D, and Hoagland, R.E. (2016) Rapid Kudzu Eradication and Switchgrass Establishment through Herbicide, Bioherbicide and Integrated Programs. *Biocontrol Science and Technology*, **26**, 640-650. https://doi.org/10.1080/09583157.2016.1141175

[26] Boyette, C.D., Reddy, K.N. and Hoagland, R.E. (2006) Glyphosate and Bioherbicide Interaction for Controlling Kudzu (*Pueraria lobata*), Redvine (*Brunnichia ovata*), and Trumpet Creeper (*Campsis radicans*). *Biocontrol Science and Technology*, **16**, 1067-1077. https://doi.org/10.1080/09583150600828742

[27] Boyette, C.D., Hoagland, R.E., Weaver, M.A. and Reddy, K.N. (2008a) Redvine (*Brunnichia ovata*) and Trumpetcreeper (*Campsis radicans*) Controlled under Field Conditions by a Synergistic Interaction of the Bioherbicide *Myrothecium verrucaria* and Glyphosate. *Weed Biology and Management*, **8**, 39-45. https://doi.org/10.1111/j.1445-6664.2007.00272.x

[28] Mortimer, P.H., Campbell, J., DiMenna, M. and White, E.P. (1971) Experimental Myrotheciotoxicosis and Poisoning in Ruminants by Verrucarin A and Roridin A. *Research in Veterinary Science*, **12**, 508-515. https://doi.org/10.1016/S0034-5288(18)34104-3

[29] Jarvis, B.B., Pavanasasivam, G. and Bean, G.A. (1985) Mycotoxin Production from *Myrothecium* Species. In: Lacey, J., Ed., *Trichothecenes and Other Mycotoxins*, J. Wiley, New York, 221-231.

[30] Abbas, H.K., Johnson, B.B., Shier, W.T., Tak, H., Jarvis, B.B. and Boyette, C.D.

(2002) Phytotoxicity and Mammalian Toxicity of Macrocyclic Trichothecene Mycotoxins from *Myrothecium verrucaria*. *Phytochemistry*, **59**, 309-313. https://doi.org/10.1016/S0031-9422(01)00464-2

[31] Hoagland, R.E., Boyette, C., Douglas, Weaver, Mark, A. and Abbas, H.K. (2007) Bioherbicides: Research and Risks. *Toxin Reviews*, **26**, 313-342. https://doi.org/10.1080/15569540701603991

[32] Boyette, C.D., Weaver, M.A., Hoagland, R.E. and Stetina, K.C. (2008) Submerged Culture of a Mycelial Formulation of a Bioherbicidal Strain of *Myrothecium verrucaria* with Mitigated Mycotoxin Production. *World Journal of Microbiology and Biotechnology*, **24**, 2721-2726. https://doi.org/10.1007/s11274-008-9759-6

[33] Boyette, C.D. and Hoagland, R.E. (2007) Evaluation of the Bioherbicide *Myrothecium verrucaria* for Weed Control in Tomato (*Lycopersicon esculentum*). *Biocontrol Science and Technology*, **17**, 171-178. https://doi.org/10.1080/09583150600937451

[34] Hoagland, R.E., McCallister, T.S., Boyette, C.D., Weaver, M.A. and Beecham, R.V. (2011) Effects of *Myrothecium verrucaria* on Morning-Glory (*Ipomoea*) Species. *Allelopathy Journal*, **27**, 151-162.

[35] Hoagland, R.E., Teaster, N.D. and Boyette, C.D. (2013) Bioherbicidal Effects of *Myrothecium verrucaria* on Glyphosate-Resistant and -Susceptible Palmer Amaranth Biotypes. *Allelopathy Journal*, **31**, 367-376.

[36] Hoagland, R.E., Boyette, C.D. Jordan, R.H. and Stetina, K.C. (2018) Interaction of the Bioherbicide *Myrothecium verrucaria* with Technical-Grade Glyphosate on Glyphosate-Susceptible and -Resistant Palmer Amaranth. *American Journal of Plant Sciences*, **9**, 2306-2319. https://doi.org/10.4236/ajps.2018.911167

[37] Steele, R.G.D., Torrey, J.H. and Dickeys, D.A. (1997) Multiple Comparisons. In: *Principles and Procedures of Statistics*: *A Biometrical Approach*, McGraw Hill, New York, 666 p.

[38] Colby, S.R. (1967) Calculating Synergistic and Antagonistic Responses of Herbicide Combinations. *Weeds*, **15**, 20-22. https://doi.org/10.2307/4041058

[39] Gisi, U., Binder, H. and Rimbach, E. (1985) Synergistic Interactions of Fungicides with Different Modes of Action. *Transactions of the British Mycological Society*, **85**, 299-306. https://doi.org/10.1016/S0007-1536(85)80192-3

[40] Sharon, A., Amsellem, Z. and Gressel, J. (1992) Glyphosate Suppression of an Elicited Defense Response. *Plant Physiology*, **98**, 654-659. https://doi.org/10.1104/pp.98.2.654

[41] Peng, G. and Byer, K.N. (2005) Interactions of *Pyricularia setariae* with Herbicides for Control of Green Foxtail (*Setaria viridis*). *Weed Technology*, **19**, 589-598. https://doi.org/10.1614/WT-04-130R.1

[42] Mitchell, J.K., Yerkes, C.N., Racine, S.R. and Lewis, E.H. (2008) The Interaction of Two Potential Fungal Bioherbicides and a Sub-Lethal Rate of Glyphosate for the Control of Shattercane. *Biological Control*, **46**, 391-399. https://doi.org/10.1016/j.biocontrol.2008.02.009

[43] Peng, G. and Wolf, T.M. (2011) Herbicide-Microbial Synergy for Improved Weed Control. *Pest Technology*, **5**, 18-27.

[44] Boyette, C.D., Hoagland, R.E. and Weaver, M.A. (2008) Interaction of a Bioherbicide and Glyphosate for Controlling Hemp Sesbania in Glyphosate-Resistant Soybean. *Weed Biology and Management*, **8**, 18-24. https://doi.org/10.1111/j.1445-6664.2007.00269.x

[45] Weaver, M.A., Jin, X., Hoagland, R.E. and Boyette, C.D. (2009) Improved Bioherbicidal Efficacy by *Myrothecium verrucaria* via Spray Adjuvants or Herbicide Mixtures. *Biological Control*, **50**, 150-156. https://doi.org/10.1016/j.biocontrol.2009.03.007