



Improved bioherbicidal efficacy by *Myrothecium verrucaria* via spray adjuvants or herbicide mixtures

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ABSTRACT

Herbicides and spray adjuvants were evaluated for compatibility with the bioherbicidal fungus, *Myrothecium verrucaria*. Several commercial formulations of glyphosate were found to be compatible for tank mixing with *M. verrucaria*, including Touchdown[®] and RoundUp HiTech[®]. Others, such as Accord XRT II[®] and RoundUp WeatherMAX[®] killed all the spores of *M. verrucaria* immediately after mixing at only 10% the maximum labeled application rate. Many herbicides, which were not suitable for co-application with *M. verrucaria*, did not inhibit the growth of the fungus when added directly to media at up to 1% concentration, indicating that these products could be compatible with *M. verrucaria* as sequential applications in an integrated weed management system. Several commercially available spray adjuvants and polyoxyethylene tridecyl ether (TDA) formulations were tested *in vitro* for their efficiency in dispersing spores and in a plant bioassay for bioherbicidal activity. All of the products improved the activity of *M. verrucaria* over the water-only treatments and TDA formulations with a hydrophilic–lipophilic balance (HLB) number of 8 or 10 had the highest activity. The mechanism for improved bioherbicidal activity with these adjuvants was investigated *in vitro*, and TDA HLB 8 and 10 did not significantly improve conidia dispersal or accelerate spore germination relative to other surfactants. It is possible that the role of the surfactant is in the alteration of the plant cuticle or otherwise preparing the infection court. Better adjuvant selection and integration with affordable synthetic herbicides should aid in the development of more cost-effective biological control of weeds.

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1. Introduction

Myrothecium verrucaria is a unique biological control agent. This fungus is a highly effective pathogen of several important annual and perennial weed species such as redvine (*Brunnichia ovata* [Walt.] Shinnery), trumpet creeper (*Campsis radicans* [L.] Seem. ex Bureau), redroot pigweed (*Amaranthus retroflexus* L.) and several morning-glory species (*Ipomea* spp.) (Walker and Tilley, 1997; Millhollon et al., 2003; Boyette et al., 2006). Several legume species are attractive targets for *M. verrucaria*, including kudzu (*Pueraria montana* var. *lobata* (Willd.) Maesen & S.M. Almeida), hemp sesbania (*Sesbania exaltata* [Raf.] Rydb. ex A.W. Hill) and sicklepod (*Senna obtusifolia*, [L.] Irwin & Barneby) (Hoagland et al., 2007).

Unlike most classical biological control agents, *M. verrucaria* does not spread well in field conditions, so it must be mass-produced, formulated, and applied directly to the weed targets (Walker and Tilley, 1997). This inability to cause secondary infections could be viewed as a safety feature, by preventing off-site move-

ment and non-target effects. The necessity to produce and apply the pathogen, however, is a substantial expense and a potential impediment to general acceptance of the bioherbicide. Consequently, lowering the cost of pathogen production, or decreasing the pathogen application rate while still achieving effective weed control are priorities in bioherbicide development.

One means of improving pathogen efficacy is through selection and addition of optimized spray adjuvants. Adjuvants, including surfactants, stickers, sun screen agents, humectants, anti-evaporation agents and even micro-nutrients (Prasad, 1993) are compounds that may improve the efficacy of mycoherbicides through more uniform pathogen distribution, better propagule adhesion to the target weed or through alteration of the waxes on the leaf surface. Studies on adjuvants have mainly focused on their role in spore germination, mycelial growth, and formation of appressoria (Prasad, 1994; Zhang et al., 2003; Bailey et al., 2004). There are numerous publications exploring the potential to improve the bioherbicidal potential of various pathogens through application in oil and water inversions (Boyette, 2006; Boyette et al., 2007a,b; Quimby et al., 1988; Shabana, 2005). Others have improved efficacy through addition of surfactants (e.g., Wyss et al., 2004; Zhang et al., 2003). Some of these surfactant trials have been quite

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extensive, as in the case of the 16 adjuvants evaluated by Wyss et al. (2004).

In spite of all these trials, there is no practical way to test all of the numerous adjuvants commercially available and, at present, there are no general guidelines to assist in the optimization of compatible adjuvants for mycoherbicide formulations (Zhang et al., 2003). Further optimization studies of compatible adjuvants are needed in relation to formulation processes. Non-ionic surfactants consist of a molecule that possesses both hydrophilic and lipophilic groups (or polar and non-polar groups), and it is the size and strength of these two groups that is called the hydrophilic–lipophilic balance number (HLB) (Griffin, 1949, 1954). Calculation of the HLB value for a non-ionic surfactant was established by Griffin (1954). In Griffin's system, a surfactant that is lipophilic in character is assigned a low HLB number and a surfactant that is hydrophilic in character is assigned a high HLB number. Griffin's method is satisfactory for non-ionic surfactants of various chemical groups. Jin et al. (1999, 2008) applied HLB as a guideline in optimizing a compatible non-ionic surfactant in formulation development of an hydrophobic conidia-based mycoinsecticide. However, no studies have established guidelines for adjuvant selection in the development of mycoherbicides.

An additional concern unique to *M. verrucaria* is the association with several mycotoxins, the macrocyclic trichothecenes. While a recent publication has demonstrated methods to produce conidia of *M. verrucaria* with greatly reduced trichothecene levels (Weaver et al., 2009), it is, to date, unproven if conidia produced in this system would still be bioherbicidal.

1.1. Integrated bioherbicide control

Another means of improving efficacy and reducing cost is to co-apply the bioherbicide with inexpensive synthetic herbicides. While the pathogen and the herbicide could be delivered sequentially (Smith and Hallett, 2006; Mitchell et al., 2008) it would be a great advantage to apply them simultaneously.

Research to date with *M. verrucaria* has generally included the surfactant Silwet L-77 (e.g., Boyette et al., 2008b) or oil–water emulsions (Millhollon et al., 2003). Numerous other surfactants are available and recently a systematic, quantitative means of evaluating surfactants based on the hydrophilic–lipophilic balance (HLB) was proposed for biological control agents (Jin et al., 2008). Similarly, there are many commercially available synthetic herbicides that could be useful to extend the weed control spectrum or to improve the efficacy of a biological control agent. Glyphosate is a very widely used, effective and reasonably priced herbicides and many bioherbicide programs have included work on compatibility with glyphosate (Boyette et al., 2008a,b). In some cases, however, the biological control agent was not found to be compatible with the available glyphosate formulations (Smith and Hallett, 2006; Weaver and Lyn, 2007).

1.2. Objectives

The objectives of the present study were to verify the pathogenicity of the reduced trichothecene formulation of *M. verrucaria* spores; measure the survival of conidia of *M. verrucaria* in tank mix suspensions of commercially formulated herbicides; assess the possible toxic effects of herbicides on the growth of *M. verrucaria*; evaluate the bioherbicidal activity of *M. verrucaria* when formulated with various surfactants; and to determine the efficacy of several surfactants in dispersing and promoting the germination of conidia of *M. verrucaria*. Results generated from these objectives would aid in the establishment of guidelines for the optimization of bioherbicidal surfactants.

2. Materials and methods

2.1. Production of *Myrothecium verrucaria* conidia

Cultures of *M. verrucaria* (IMI 361390) were grown on potato dextrose agar (PDA) or a Vogel's-based, defined agar medium containing 15 g L⁻¹ glucose (Weaver et al., 2009) under a 12 h light–dark cycle. Cultures were scraped with a transfer pipette under deionized water to collect conidia. Aliquots of the spore suspensions were mixed with ethanol (1:1 vol/vol) for HPLC separation, detection and quantification of trichothecene mycotoxins (Weaver et al., 2009). After preliminary experiments demonstrated the mitigation of mycotoxins through use of the Vogel's medium, it was used for all further experiments.

2.2. Viability of *Myrothecium verrucaria* conidia with herbicide mixtures

Commercial formulations of herbicides used in this study are detailed in Table 1. Maximum use rates were determined by the manufacturer's label guidance, and based upon a 374 L ha⁻¹ application volume. Some of the herbicide labels indicate a requirement for the addition of 0.25% surfactant, and since the bioherbicidal activity of *M. verrucaria* is enhanced by Silwet L-77, it was included in all treatments. Fresh stock suspensions of the herbicides were prepared before each experiment. Sterile centrifuge tubes were prepared with *M. verrucaria* spore suspensions and Silwet L-77 (0.25% vol/vol) in water. After a brief pre-incubation to disperse the spores, herbicide solutions were added from the stock solutions to yield the maximum labeled application rate, 1× (Table 1), or 0.5× or 0.1× (vol/vol). Each herbicide-rate treatment was created in four tubes and the entire experiment was repeated entirely three times. Maximum rates were calculated assuming 374 L ha⁻¹ application volume. Aliquots were removed immediately and after 3, 6, 26 and 50 h of incubation while being constantly shaken on a rotary incubated shaker (125 RPM, 27 °C). These aliquots were serially diluted and mixed with molten agar (49 °C) to determine the number of remaining viable colony forming units (CFUs). Percent survival was calculated by comparison to a herbicide-free treatment.

2.3. *In vitro* growth inhibition of *Myrothecium verrucaria*

Spore suspensions of *M. verrucaria* were placed in the center of Petri dishes containing PDA supplemented with up to 1% concentration of commercially available herbicide formulations. The colony diameter was measured every 1–3 days thereafter for 7 days. A linear growth rate was fitted to the measured diameters and the growth rates were normalized by comparison to growth on unamended PDA. Each herbicide treatment and rate was observed on five plates. Results were used to repeat the experiment with different rates until a concentration that yielded 50% inhibition of growth could be bracketed. All herbicides were evaluated in at least three experiments.

2.4. Bioassay of *Myrothecium verrucaria* bioherbicidal activity

Sicklepod (*S. obtusifolia*) seedlings were grown to the two true leaf stage (ca. 10 cm) in potting mix (Jiffy Mix, Jiffy Products, Batavia, IL 60510, USA). Adjuvants were added to stock suspensions of *M. verrucaria* conidia to yield 0.25% adjuvant concentration and spore concentrations of 1 × 10⁸ and 2 × 10⁷ spores mL⁻¹. Four individual plants were sprayed with these mixtures with a hand-held trigger-action sprayer to run-off (ca. 400 L ha⁻¹). After 5 days the plants were visually rated on a disease severity scale (1, dead

Table 1
Commercial formulations of herbicides and adjuvants evaluated for compatibility with *M. verrucaria*.

Product name	Supplier	Active ingredient	Classification/mode of action	Maximum use rate (as formulated)	Application rate (%) ^a
Touchdown ^{®b}	Syngenta	Glyphosate–diammonium salt	EPSP synthase inhibitor	9.34 L ha ⁻¹	2.5 ^c
Touchdown Total [®]	Syngenta	Glyphosate–potassium salt	EPSP synthase inhibitor	7.95 L ha ⁻¹	2.1
Touchdown HiTech [®]	Syngenta	Glyphosate–potassium salt	EPSP synthase inhibitor	7.02 L ha ⁻¹	1.8
Accord XRT II [®]	Dow AgroScience	Glyphosate–dimethylamine salt	EPSP synthase inhibitor	18.71 L ha ⁻¹	2.5
RoundUp Ultra [®]	Monsanto	Glyphosate–isopropyl amine salt	EPSP synthase inhibitor	18.71 L ha ⁻¹	2.5
RoundUp WeatherMAX [®]	Monsanto	Glyphosate–potassium salt	EPSP synthase inhibitor	7.02 L ha ⁻¹	1.9
Milestone [®]	Dow AgroScience	Aminopyralid	Synthetic auxin	0.51 L ha ⁻¹	0.14
Vista [®]	Dow AgroScience	Fluroxypyr	Synthetic auxin	3.12 L ha ⁻¹	0.8
Transline [®]	Dow AgroScience	Clopyralid	Synthetic auxin	1.56 L ha ⁻¹	0.4
PastureGard [®]	Dow AgroScience	Fluroxypyr	Synthetic auxin	9.35 L ha ⁻¹	2.5
Tordon K [®]	Dow AgroScience	Picloram	Synthetic auxin	4.68 L ha ⁻¹	1.3
Tordon 101 [®]	Dow AgroScience	Picloram + 2,4-dichlorophenoxy acetic acid	Synthetic auxin	18.71 L ha ⁻¹	5
Escort [®]	DuPont	Metsulfuron methyl	Acetolactate synthase inhibitor	242 g ha ⁻¹	0.03 ^d
UltraBlazer	BASF	Acifluorfen	Protoporphyrinogen oxidase inhibitor	1.75 L ha ⁻¹	0.5
TopSurf	Agrilience LLC	Alkylpolyethoxyalkylene ethers and their ethoxylated derivatives	Spray adjuvant		0.25
Silwet L-77	Loveland Industries	Polyalkyleneoxide modified heptamethylsiloxane	Spray adjuvant		0.25
Latron AG-98	Rohm and Haas	Alkylaryl polyoxyethylene glycols	Spray adjuvant		0.25
TopFilm SE	Biosorb Inc.	Grain derived adjuvant and emulsifiers	Spray adjuvant		0.25
Induce		Proprietary blend of alkyl aryl polyoxylkane ethers, free fatty acids and dimethyl poly siloxane	Spray adjuvant		0.25
TDA	Ethox	Polyoxyethylene tridecyl ether	Spray adjuvant		0.25

^a Application rate for foliar application with a total volume of 345 L ha⁻¹.

^b Formulation no longer commercially available.

^c Volume:volume.

^d Mass:volume.

plants; 2, no living leaves, some green stems; 3, lesions or limited necrosis; 4, healthy plants). The experiment was repeated three times. Similar results were obtained across the experiments. After a square root, arcsin transformation to normalized the variance, the bioherbicidal activity observations were analyzed via the proc mixed function of SAS v9.1 (SAS Institute, Cary, NC, USA) and results were back-transformed to the original ratings for presentation.

2.5. Conidia suspension and germination

Commercial spray adjuvants (Table 1) and polyoxyethylene tridecyl ether (TDA) (Ethox Chemical, LLC, Greenville, SC, USA) with a HLB numbers of 8, 10, 12, and 14.5 (Griffin, 1949, 1954), were evaluated for differences in dispersing *M. verrucaria* conidia, as in Jin et al. (2008). A stock suspension of conidia was quantified by hemacytometer before aliquots were serially diluted in 0.25% solutions of various surfactants. These treatments were then agitated for 30 min before spreading aliquots with approximately 100 conidia onto PDA plates. Resulting colonies were counted after 48 h incubation. A separate test measured the role the surfactants had on conidial germination. Conidia were spread on PDA plates supplemented with 0.25% concentrations of the various surfactants. After 6, 8, and 10 h the plates were inspected microscopically to measure the percentage of spore germination. Percent germination was normalized against a surfactant-free treatment and the treatment means were separated by least squares difference (SAS v. 9.1).

3. Results and discussion

3.1. Production of *Myrothecium verrucaria* conidia

Production of *M. verrucaria* on defined medium instead of PDA resulted in a dramatic decrease in mycotoxin associated with the

spores (Fig. 1). Verrucaric acid is a known phytoxin, and there is evidence that unidentified metabolites of *M. verrucaria* are involved in the observed bioherbicidal activity (Anderson and Hallett, 2004). The results presented here, however, suggest that *M. verrucaria* formulations can be prepared with greatly improved safety while maintaining bioherbicidal activity.

3.2. Viability of *Myrothecium verrucaria* conidia with herbicide mixtures

One form of chemical compatibility is the tolerance of the bioherbicides to survive in a tank mix suspension with various herbicides. Examples of chemical incompatibility with bioherbicides are well documented. For example, *Microshpaeropsis amaranthi* was shown to be incompatible with several commercially available glyphosate products (Smith and Hallett, 2006), although the incompatibility appeared to be the result of the commercial formulations rather than the glyphosate *per se*. We have previously reported on synergistic interactions of glyphosate and *M. verrucaria* when co-applied (Boyette et al., 2008b) but have noted that not all commercially available products were compatible with *M. verrucaria* (Boyette et al., 2006). In this context it is necessary to test for compatibility with commercially available products rather than simply the active ingredient. Table 2 details the survival of *M. verrucaria* conidia in simulated tank mix suspensions of herbicides. Some herbicides, such as Escort[®], PastureGard[®] and several glyphosate-based products caused immediate, large losses in viability (Table 2). Others, such as Milestone[®], seemed much more compatible with *M. verrucaria* and high viability was retained even after prolonged exposure. Some of decline could be attributed to aggregation of conidia, even in the presence of surfactants and with constant shaking, but more generally is a result of the toxicity of the herbicides on *M. verrucaria*. In the case of many herbicides, the toxicity cannot be readily partitioned to the active ingredient or to the various carriers and adjuvants. In the case of the glyphosate-based

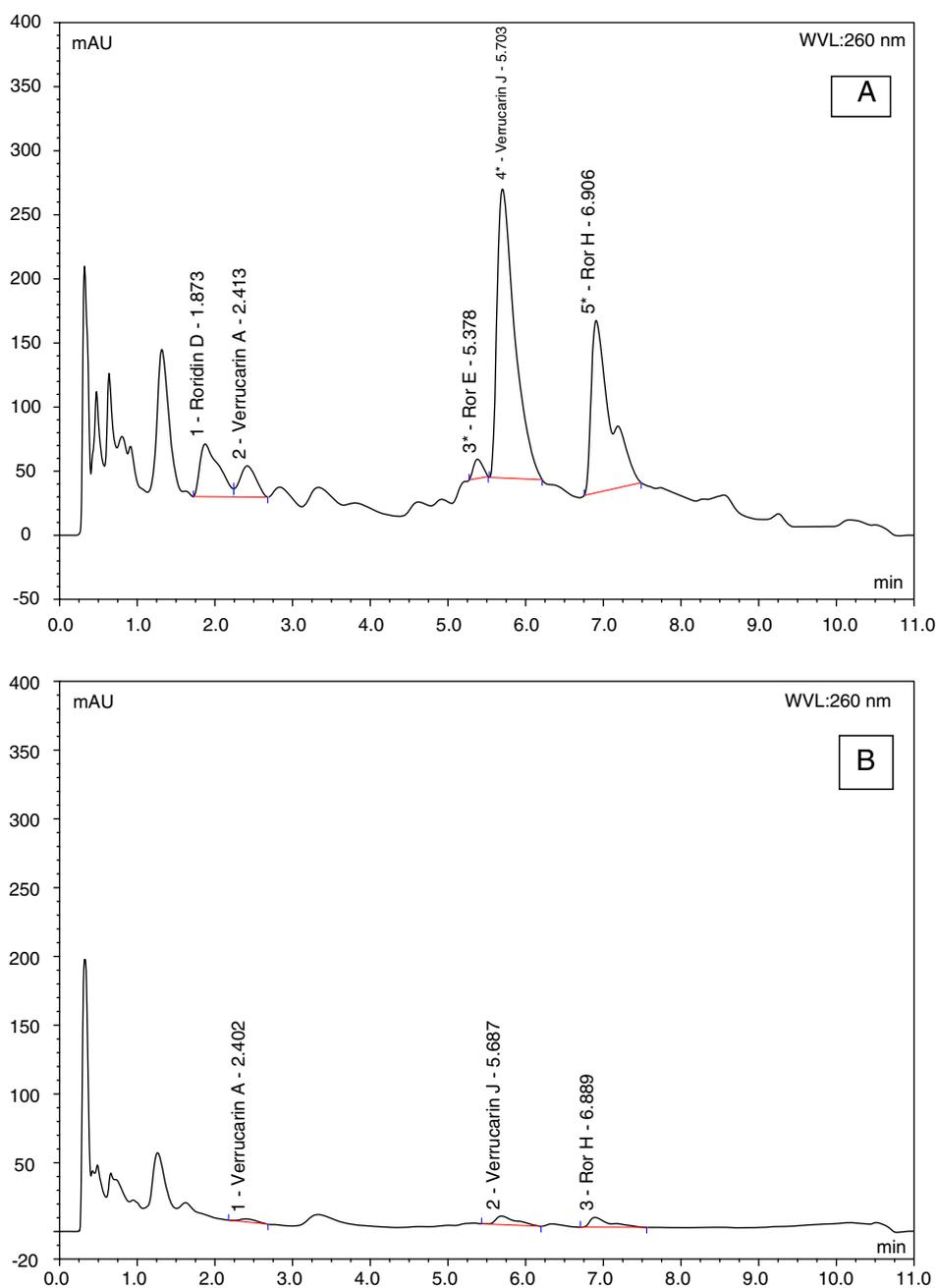


Fig. 1. HPLC chromatograph of *M. verrucaria* extracts. (A) Detection of macrocyclic trichothecenes in an extract of *M. verrucaria* grown for 15 days on potato dextrose agar. (B) Detection of macrocyclic trichothecenes in an extract of *M. verrucaria* grown for 7 days on a defined Vogel's and glucose agar medium.

herbicides, however, the high level of tolerance to RoundUp HiTech[®] and Touchdown[®] and the dramatic incompatibility with RoundUp WeatherMAX[®] indicates that the active ingredient is not particularly toxic at the rates used here.

3.3. *In vitro* growth inhibition of *Myrothecium verrucaria*

The other form of chemical compatibility is the ability of the bioherbicide to grow in the presence of agrochemicals. If it is not possible to co-apply *M. verrucaria* with a particular synthetic herbicide, it might be possible to use the two sequentially. In that case, the ability of *M. verrucaria* to grow in the presence of these products would be a concern. The inhibitory effect on *M. verrucaria* of several widely used herbicides is indicated in Table 3. While we were able to measure a dose-dependent reduction in the growth

rate of *M. verrucaria* in response to these herbicides, none of these products were significantly inhibitory at the low rates likely to be encountered in a foliar application.

There are several reasons for combining bioherbicides with sub-lethal rates of synthetic herbicides. The chief is economic. Regardless of improvements in bioherbicide production, the cost of the biological agent is likely to be greater than that of many chemical herbicides. In some cases, a synergistic effect between the biological agent and the chemical herbicide could provide reasonably priced, effective weed control. Such synergy has been demonstrated for annual weeds in row crops (Boyette et al., 2008a), perennial broadleaf weeds (Boyette et al., 2008b), and with a pathogen mixture supplemented with glyphosate for a grassy weed (Mitchell et al., 2008). While some have questioned the responsibility of using sub-lethal rates of herbicides, the risks of herbicide

Table 2
Survival of *M. verrucaria* in herbicide tank mixes.

Product/mixing rate	0 h	3 h	6 h (% survival)	26 h	50 h
<i>Accord</i>					
1× ^a	0	0	0	0	0
0.5×	0	0	0	0	0
0.1×	0	0	0	0	0
<i>RoundUp WeatherMAX</i>					
1×	0	0	0	0	0
0.5×	0	0	0	0	0
0.1×	0	0	0	0	0
<i>Touchdown total</i>					
1×	47	28	0	0	0
0.5×	74	44	0	0	0
0.1×	100	69	11	0	0
<i>Touchdown HiTech</i>					
1×	57	57	45	23	23
0.5×	78	60	57	36	23
0.1×	100	53	58	32	22
<i>Tordon</i>					
K 1×	5	0	0	0	0
0.5×	13	0	0	0	0
0.1×	24	3	0	0	0
<i>PastureGard</i>					
1×	1	0	0	0	0
0.5×	5	0	0	0	0
0.1×	32	0.5	0	0	0
<i>Vista</i>					
1×	9	0	0	0	0
0.5×	19	0	0	0	0
0.1×	83	13	0	0	0
<i>Milestone</i>					
1×	94	89	85	63	50
0.5×	94	90	88	70	54
0.1×	96	92	88	75	59
<i>Escort</i>					
1×	5	0	0	0	0
0.5×	13	0	0	0	0
0.1×	24	3	0	0	0
<i>Transline</i>					
1×	33	2	0	0	0
0.5×	20	3	4	0	0
0.1×	33	32	30	12	2

^a 1× refers to the maximum application rate, as listed in Table 1 assuming a 374 L ha⁻¹ application volume. 0.5× and 0.1× indicate one half and one tenth the maximum rate, respectively.

Table 3
Inhibition of radial growth of *M. verrucaria* by herbicides and adjuvants.

Commercial formulation	IC ₅₀ ^a
<i>Glyphosate-based herbicides</i>	
Touchdown	0.6%
Accord XRT II	0.55%
Touchdown HiTECH	>2%
Touchdown Total	0.55%
Roundup Ultra	0.35%
Roundup WeatherMAX	0.3%
<i>Other herbicides</i>	
Vista	0.3%
Milestone	>3%
Transline	>3%
Tordon	>3%
Tordon 101	>3%
UltraBlazer	0.45%
Escort	0.4%
PastureGard	0.8%
<i>Adjuvants</i>	
TopSurf	>1%
Induce	4%

^a IC₅₀ expressed on a percentage basis vol:vol, except for Escort, which is on a mass:vol basis.

resistance might be mitigated by combining the two modes of action, thus delaying the local development of a herbicide-resistant weed population. Kudzu (*P. montana* var. *lobata*) is a principal target weed for *M. verrucaria* bioherbicide research. Current herbicides most commonly recommended for kudzu control are acetolactate synthase inhibiting herbicides and synthetic auxins, which are the first and fourth highest ranking groups of herbicides with resistant weed populations, respectively, (Heap, 2009). If the bioherbicide and synthetic herbicide are combined appropriately then the two modes of action could combine to yield a lethal dose without contributing to the development of herbicide resistance.

3.4. Bioassay of *Myrothecium verrucaria* bioherbicide activity

Selection of adjuvant had a significant effect on the plant disease ratings and on the plant dry weights (Table 4). While Silwet L-77 has been used with *M. verrucaria*, it was the least effective adjuvant at the tested concentrations in the bioassay against sicklepod. Of the commercially available adjuvants tested, Induce, Latron AG-98 and TopFilm provided the highest level of bioherbicide activity, depending on the concentration. The activity of TopFilm was especially noteworthy as this is a grain-derived product. Use of this or a similar product might facilitate the use of *M. verrucaria* in organic crop production. In addition to tests with the commercial adjuvants, which include proprietary components, we also tested TDA adjuvants with a series of HLB values. In general, TDA formulations with lower HLB numbers resulted in better bioherbicide efficacy, especially at low inoculum concentration. The best

Table 4
Bioherbicide activity of *M. verrucaria* against sicklepod seedlings.

Surfactant	Disease rating ^a (high inoculum ^b)	Disease rating (low inoculum)
None	3.51 (a) ^c	3.89 (a)
Induce	1.29 (cde)	1.84 (de)
Latron AG-98	1.54 (bcd)	2.24 (cd)
Silwet L-77	2.06 (b)	3.08 (b)
TopFilm	1.06 (e)	2.49 (bcd)
TopSurf	1.84 (bc)	2.93 (bc)
TDA HLB 8	1.14 (de)	1.48 (e)
TDA HLB 10	1.05 (e)	1.84 (de)
TDA HLB 12	1.33 (cde)	2.03 (de)
TDA HLB 14.5	1.48 (bcde)	2.87 (bc)
Uninoculated	4	

^a Disease ratings based on a 4-point visual disease severity scale from 1 (dead plants) to 4 (healthy plants). Individual plants were scored. Data are from 3 replicates of 4 plants each.

^b High and low inocula correspond to 1 × 10⁸ and 2 × 10⁷ spores mL⁻¹, respectively.

^c Within a column values with the same letter are not significantly different at 0.05 level.

Table 5
Colony forming units after *M. verrucaria* conidia were suspended in 0.25% surfactant solutions.

Surfactant ^a	CFU/plate	Mean separation ^b
TDA HLB 14.5	75.2	A
Silwet L-77	72.8	A
TDA HLB 12	63	B
TDA HLB 8	55.3	B, C
TDA HLB 10	51.7	C
Induce	50.2	C
TopFilm	15.5	D
Water	10.8	D

^a Complete descriptions of surfactants provided in Table 1.

^b Treatment with the same letter are not significantly different at the alpha = 0.05 level after mean separation.

Table 6
Germination of conidia of *M. verrucaria* after suspension in 0.25% surfactant solutions.

6 h Incubation			8 h Incubation		10 h Incubation	
Surfactant ^a	Germination (%)	Mean separation	Germination (%)	Mean separation	Germination (%)	Mean separation
Water	30	A ^b	72.7	A, B	88	A
TopFilm	29.1	A, B	72.9	A	87.7	A, B
Induce	27	A, B, C	67.1	A, B	83.9	B
TDA HLB 14.5	26.5	A, B, C, D	70	A, B	86.7	A, B
TDA HLB 12	25.9	B, C, D	65.8	A, B	86.5	A, B
Silwet L-77	25.3	B, C, D	68.1	A, B	85.5	A, B
TDA HLB 10	25	C, D	64.4	B	85.2	A, B
TDA HLB 8	22.7	D	64.8	B	86.5	A, B

^a Complete descriptions of surfactants provided in Table 1.

^b Treatment with the same letter are not significantly different at the alpha = 0.05 level after mean separation.

bioherbicidal activity was associated with TDA formulations with an HLB of 8–10, although statistically similar results at high inoculum concentrations were found with HLB 12.

3.5. Conidia suspension and germination

There are several possible mechanisms by which spray adjuvants can improve the bioherbicidal activity of *M. verrucaria*. Conidia of *M. verrucaria* are slightly hydrophobic and the addition of a surfactant could aid in wetting and dispersal of the conidia in the spray mixture. After deposition on the phyloplane, different surfactants could have varying effects on the rate of conidia germination. Finally, the surfactants could facilitate an infection court that is more favorable to *M. verrucaria* by alteration of the host plant cuticle. The first two mechanisms were investigated through *in vitro* tests. First, the surfactants were evaluated for the ability to disperse conidia, resulting in differences in CFU on PDA plates. While significant differences were observed (Table 5), the results were quite different than the bioherbicidal bioassay. For example, while TDA 8 and 10 were most effective in promoting the bioherbicidal activity, they were not especially effective in increasing the CFU *in vitro*. Although Silwet L-77 was the least effective adjuvant in the bioassay, it was among the best in dispersing the conidia. The other *in vitro* test of surfactants was an observation of the conidia germination rates after dilution and brief incubation in surfactant. These results (Table 6) also were inconsistent with the observations from the greenhouse bioassay. Surfactants TDA 8 and 10 yielded among the best bioherbicidal results, but generally among the slowest conidia germination rates. While almost no bioherbicidal activity was found without any surfactant (Table 4), this treatment had the highest percent germination rate at all observed time points. In general, the *in vitro* tests did not support the hypothesis that the spray adjuvants improved the activity of *M. verrucaria* on sicklepod though spore dispersal or by promoting more rapid spore germination. Thus, the role of the adjuvants is more likely to be in changing the physical/chemical characteristics of the leaf surface and the spore–leaf interactions. Griffin (1949) indicated that surfactants with lower HLB numbers (4–6) were mostly used as emulsifiers (water-in-oil), while those with higher HLB numbers (13–15) were detergents, and surfactants with HLB numbers between 7 and 9 were superior candidates for wetting agents. Better wetting might play a role in alteration of the host plant cuticle, and therefore facilitate the infection process. Surfactants with higher HLB numbers, in the range of detergents, are prone to foaming. Foam formation should be avoided because it reduces the uniformity of the conidial suspension, and further reduces the bioactivity of bioherbicides (Table 4). However, surfactants in the range of detergents have better dispersion ability and may result in higher CFU counts (Table 5). Lower HLB number surfactant did have a lower germination rates in 6 h, but by 10 h

the difference in germination became statistically non-significant (Table 6). Early events in *M. verrucaria* pathogenesis are not well-understood, so it is unclear if this early, transient difference in spore germination would be biologically meaningful.

4. Conclusions

At present, the biological control of crop weeds is not generally an economically viable choice. Results presented here demonstrate the possibility of greatly improved weed control by *M. verrucaria* though improved selection of spray adjuvants or though integration with standard chemical control. While effective bioherbicidal activity has been demonstrated with *M. verrucaria* when formulated with Silwet L-77 (e.g., Hoagland et al., 2007), we present here that similar or improved results can be obtained with other surfactants while using only one fifth the rate of *M. verrucaria* conidia. Additionally, the demonstration of compatibility with some, but not all, commercially available glyphosate herbicides is important in the development of an integrated weed control program. Our results indicate the potential of HLB ratings as a guideline in selection of a compatible surfactant. In the selection of a surfactant for any application, we must have the correct chemical group, which means compatibility in most cases, and the optimized HLB number (Griffin, 1949). Optimized HLB number of a compatible surfactant may not only enhance bioactivity of a bioherbicide, but also improve the chemical properties of a formulation.

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