RESEARCH NOTE

Compatibility of a Biological Control Agent With Herbicides for Control of Invasive Plant Species

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ABSTRACT: Kudzu, *Pueraria montana* var. *lobata,* is an exotic invasive weed that is difficult to control with available products and management practices. The fungal pathogen, *Myrothecium verrucaria*, is being developed as a bioherbicide for kudzu and other invasive vines. This biological control agent might be applied with conventional herbicides to improve the efficacy or spectrum of weed control. The survival of *M. verrucaria* was measured over time in simulated tank-mixes of commercial formulations of the herbicides: amniopyralid (Milestone*), metsulfuron (Escort XP), and fluroxypyr (Vista). The fungus was also grown in vitro in the presence of these herbicides to evaluate any growth inhibition. *M. verrucaria* was highly tolerant to all concentrations of amniopyralid and metsulfuron for up to two days in simulated tank-mixes, while mixtures with fluroxypyr resulted in a gradual loss of spore viability. The fungus grew on media supplemented with amniopyralid and metsulfuron with only small effects on the growth rate, but fluroxypyr caused growth inhibition. These studies provide insight for developing effective, integrated control strategies for kudzu.

Index terms: augmentative biocontrol, bioherbicide, pathogen-herbicide interactions

* Mention of trade names or commercial products is solely for providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

INTRODUCTION

The history of kudzu, *Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.), in the southeastern United States is typical of many exotic invasive plant species. By the 1930s, kudzu was widely promoted and planted, with the best of intentions, by the government to control soil erosion, by homeowners as a shade plant, and by farmers who needed a well-adapted, fast-growing forage crop. However, as with many other well-adapted, fast-growing introduced species, the best of intentions were defeated by the law of unintended consequences. Today, kudzu covers approximately 3 million ha in the United States, primarily in the Southeast, but is also invasive from New York City (Frankel 1989) to Washington State (McGonigal 2006). Kudzu, an aggressive vine, is disruptive to native ecosystems, contributes to the severity of forest fires (Munger 2002), and undermines commercial forestry operations (Forseth and Innis 2004). A recent estimate placed the economic damage from kudzu at $54 million yr^{-1} from lost timber production within the state of Mississippi alone (A. Van Valkenburg, Area Forester, Mississippi Forestry Commission, pers. comm.). This impact may soon be surpassed by the economic losses inflicted by Asian Soybean rust, which overwinters on kudzu. While the current extent of kudzu establishment is immense, the 50,000 ha annual spread (Forseth and Innis 2004) is also a cause for concern.

Control of kudzu is difficult. Many of the chemical products labeled for use against kudzu (e.g., glyphosate, 2,4-D, picloram, metsulfuron, and tebuthiuron) have substantial non-target effects that preclude their use in natural areas. The level of control can be over 90%, but applications for up to 10 years are required to eradicate the weed (Nelson 2003). Many herbicides have significant use restrictions near water, on steep slopes, or on certain soil types. A new herbicide, aminopyralid, has been labeled under the EPA's Reduced Risk Pesticide Initiative for kudzu control. This product has foliar and soil residual activity and has a level of selectivity that might make it attractive for conservation sites, parks, and natural areas.

While kudzu is a 'classic' invasive plant species, it has not lent itself readily to classical biological control. Searches for biological control agents, especially insects, from kudzu's center of origin identified several candidate organisms, but none have yet resulted in safe and effective kudzu control in the United States (reviewed in Britton et al. 2002). While the search for classical biocontrol agents continues (Sun et al. 2006), an augmentative biocontrol approach has emerged. In contrast to classical biocontrol, which is dependent on an optimal (and elusive) interaction between host, pathogen, and environment, the augmentative biocontrol or bioherbicide approach makes use of direct pathogen delivery, often with multiple applications, just as one would apply an herbicide (reviewed in Hallett 2005). While the bioherbicidal approach may be considered less 'natural' and host-specific than classical biocontrol, it is by no means as artificial or broad-spectrum as many synthetic herbicides.

The fungal plant pathogen, *Myrothecium verrucaria,* is being developed as a bioherbicide of kudzu. This pathogen kills young kudzu plants and produces very rapid necrosis, even on mature, well-established kudzu stands (Boyette et al. 2001, 2002). Experiments are underway to determine what lasting effect *M. verrucaria* has on kudzu stands. In addition, this pathogen has efficacy on several other invasive weed species, but is safe on many native tree and grass species (Walker and Tilley 1997). *Myrothecium verrucaria* is a unique pathogen in that it is effective even in the absence of a dew period (Boyette et al. 2001, 2002; Abbas et al. 2002). Although *M. verrucaria* has been associated with mycotoxins, it reportedly does not produce them *in planta* (Abbas et al. 2001, 2002)

Given the need for integrated management of kudzu, it might be useful to apply a bioherbicide in concert with synthetic herbicides. We evaluated the compatibility of *M. verrucaria* with three herbicides with unique properties (Table 1). Fluroxypyr (Vista, Dow Agroscience) is marketed for invasive plant management in pine plantations and other non-crop sites, but is not labeled for control of kudzu; addition of *M. verrucaria* might provide a more useful control spectrum in specific applications. Metsulfuron (Escort, DuPont) is a widely recommended, effective herbicide for kudzu, and it might be expected that some users would deploy *M. verrucaria* with metsulfuron or as a follow-up. Aminopy-

Table 1. Selected control agents of invasive plant species in non-croplands.

Control agent	Use rates [a]	Approved sites	Weeds controlled [b]
Milestone (aminopyralid)	0.51 L / ha 123 g ae / ha 644 ppm [c]	Rangeland, permanent grass pastures, Conservation Reserve Program acres, non-cropland areas (such as roadsides), non-irrigation ditch banks, natural areas (such as wildlife management areas, wildlife openings, wildlife habitats, recreation areas, campgrounds, trailheads and trails) and grazed areas in and around these sites.	diffuse knapweed, Russian knapweed, spotted knapweed, kudzu, horseweed, lambsquarter, common ragweed, tropical soda apple, yellow star thistle, bull thistle, Canada thistle, musk thistle
Escort (metsulfuron methyl)	279 g / ha 167 g ai / ha 877 ppm	Non-crop areas, conifer and hardwood plantations, banks of dry drainage ditches certain types of unimproved turf.	Musk thistle, Old World climbing fern, Purple loosestrife, kudzu
Vista (fluroxypyr)	3.12 L / ha 560 g ae / ha 2932 ppm	Non-cropland areas including industrial sites, non-irrigation ditch bank, and rights-of way such as electrical power lines, communication lines, pipelines, roadsides and railroads including grazed areas within these sites. Pine plantations.	Kochia, morningglory, vetch, puncturevine, lantana, leafy spurge, wild carrot, blackberry
Myrothecium verrucaria			Kudzu, jimsonweed, sicklepod, hemp sesbania

[a] Maximum labeled rate; "ae" indicates acid equivalent; "ai" indicates active ingredient.

[b] Partial list of species on listed on product label or controlled in published reports

[c] Listed concentration is the concentration of active ingredient or acid equivalent in the spray mixture assuming 191L/ha application

ralid (Milestone, Dow Agroscience) is a newly available herbicide to land managers for kudzu control. We evaluated the time-dependent viability of *M. verrucaria* spores in tank-mix solutions of commercial formulations of these herbicides at three application concentrations. Additionally, the growth rate of *M. verrucaria* on media amended with the herbicides was investigated.

MATERIALS AND METHODS

Tank-mix compatibility

Spores of *M. verrucaria* from cultures grown for two weeks on Potato Dextrose Agar (PDA) were collected in water and filtered to remove mycelial fragments. Herbicides were prepared as aqueous solutions/suspensions at concentrations so that application of 191 L ha^{-1} would deliver the maximum labeled rate (1x concentration) (Table 1), one half the maximum labeled rate (0.5x), and one tenth the maximum labeled rate (0.1x). One attractive feature of *M. verrucaria* as a bioherbicide is that there is no detectable secondary spread of the agent away from the point of application, because it does not infect host plants unless it is applied with a surfactant, such as Silwet L-77 (Walker and Tilley 1997; Boyette et al. 2001). Consequently, this wetting agent was included in all tests presented here in all treatments at a rate of 0.2% (vol/vol). Spore suspensions were added to 20 mL simulated tank-mixes of these herbicides and incubated with constant agitation for 48 hrs. Aliquots were periodically removed, diluted, and mixed with molten PDA. Colony forming units were counted following 2 days of incubation.

In vitro growth inhibition

Aliquots of each herbicide were added to flasks of molten PDA and poured into Petri dishes. After the media solidified, 2 µL of a spore suspension, containing ca. 20,000 colony forming units (CFU), was added to the center of each plate. The colony diameter was measured periodically to detect inhibition of growth of the fungus caused by the herbicides.

Statistics

Experiments consisted of three replicates per treatment and repeated twice. Means and standard deviations were calculated and plotted against time.

RESULTS AND DISCUSSION

Successful control of kudzu is likely to require a sustained and integrated approach. Mechanical disruptions, such as bulldozing or mechanical mulching, leave the site vulnerable to subsequent species invasions. Tactics, such as grazing or prescribed burning, give only limited control, have poor selectivity, and are not advisable for many sites (Harrington et al. 2003; Nelson 2003; Sun et al. 2006). Because of these substantial limitations, it is likely that many kudzu control efforts will be largely herbicide-based (Miller 2003). In this environment, it is important that any potential bioherbicide be evaluated for compatibility with the herbicides in use. 'Compatibility' potentially includes many factors. One form of compatibility is the ability for spores of the bioherbicide to retain viability in a tank mix of an herbicide and any necessary spray adjuvants. In a recent meeting of foresters, park managers, concerned citizens, and weed scientists, a significant concern was the survivability of *M. verrucaria* in tank mixes with other herbicides (Weaver et al. 2006a). Preliminary tests with other herbicides and common adjuvants revealed a range of compatibility from almost immediate, complete loss of viability to tolerance of greater than 48 hours (Weaver et al. 2006b). Results presented in Figure 1 A-C indicate that the compatibility of *M. verrucaria* with tank-mix solutions is herbicide specific. Spores of *M. verrucaria* retained greater than 50% viability even after more than 48 hours in a simulated tank mix of a commercial formulation of aminopyralid (Figure 1A), even at the maximum labeled rate. The very low toxicity of aminopyralid, coupled with a surfactant effect and a degree of experimental error, lead to an experimental artifact at some time points that indicated greater than 100% survival. Metsulfuron formulations were also well-tolerated at all tested concentrations (Figure 1B). The commercial preparation of fluroxypyr, in contrast, exhibited substantial toxicity to *M. verrucaria* spores at the maximum and half-maximum labeled rates (Figure 1C). If *M. verrucaria* were to be used in concert with fluroxypyr, only very low use rates would be possible unless safer formulations could be developed.

Another component of bioherbicidal 'compatibility' is the tolerance to pesticide residues from previous treatments. With the sustained effort essential to eradicate established kudzu, a rotation of tactics might be beneficial to minimize non-target effects and prevent development of herbicide resistance. When *M. verrucaria* was grown in the presence of aminopyralid and metsulfuron, the growth rate was only marginally diminished, even in the presence of very high levels of the herbicides (Figures 2 A-B). In the case of aminopyralid, no growth inhibition was observed, even at concentrations three times higher than encountered in direct herbicide application. Metsulfuron diminished the growth rate of *M. verrucaria*, but only after four days, at which time the pathogen would have already killed the kudzu (Boyette et al. 2001, 2002). In contrast, fluroxypyr significantly reduced the fungal growth rate (Figure 2 C)

Collectively, the results presented here support the role that the bioherbicide, *M. verrucaria,* can play in controlling kudzu. Although herbicide spray mixtures are not routinely subject to long incubations, equipment failures or weather changes sometimes delay application. Even these adversities are reasonably well tolerated by spores of *M. verrucaria* with both tested herbicides that are labeled for use on kudzu. Even fluroxypyr could be used with *M. verrucaria* at low use rates or if immediate application were possible. The compatibility of *M. verrucaria* with commercial preparations of these herbicides and the growth in the presence of herbicide indicates that *M. verrucaria* could be incorporated into an integrated weed management program for kudzu.

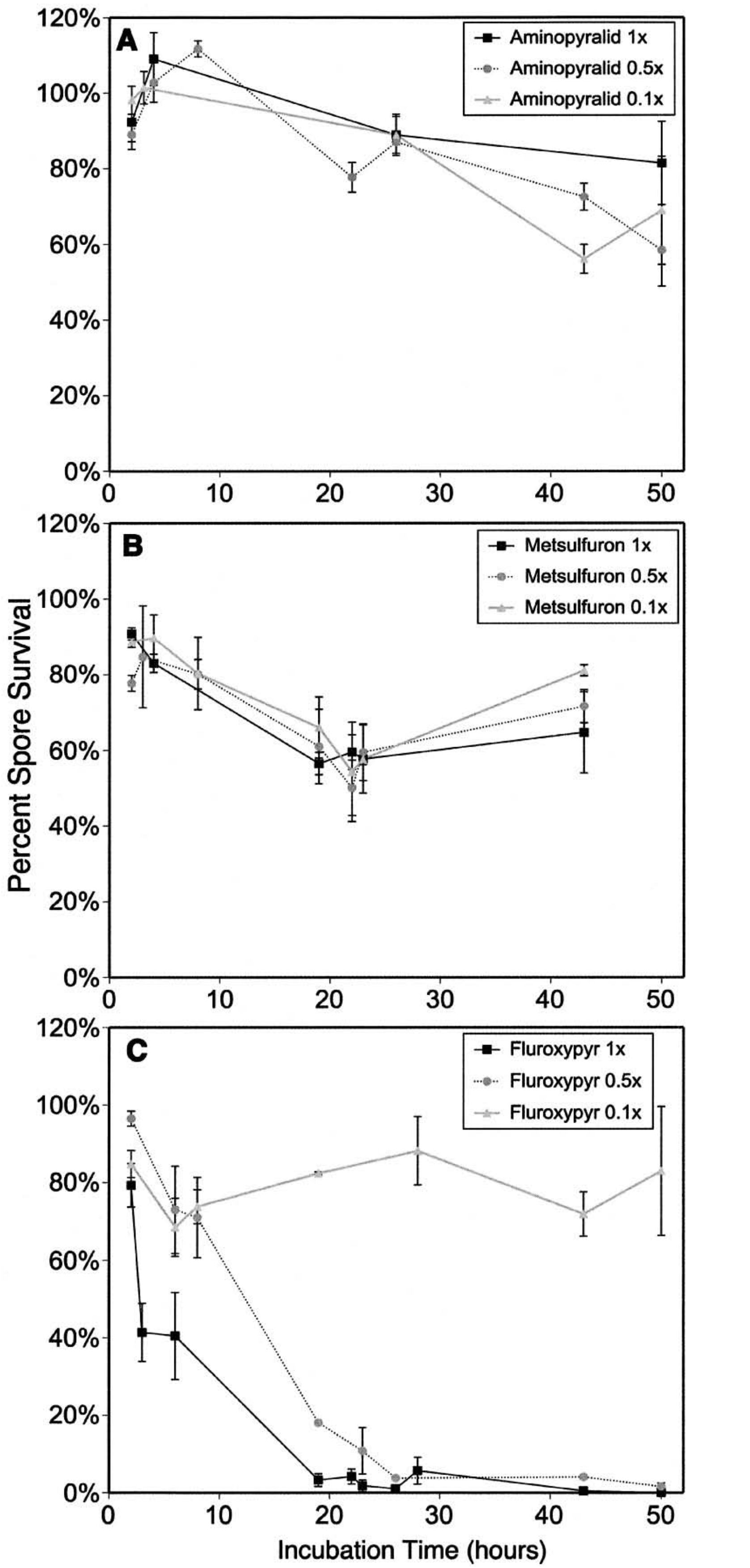

Figure 1. Viability of *M. verrucaria* in tank-mixes of herbicides.

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Margaret Lyn is a research chemist with the USDA-ARS in New Orleans, La. Her research focus is on development of microbial delivery systems for insect, weed, disease, or toxin control.

Mark Weaver's research is on the development of Myrothecium verrucaria for biological control of kudzu through strain selection and strain improvement. Other recent research includes environmental fate of herbicides and soil microbiology.

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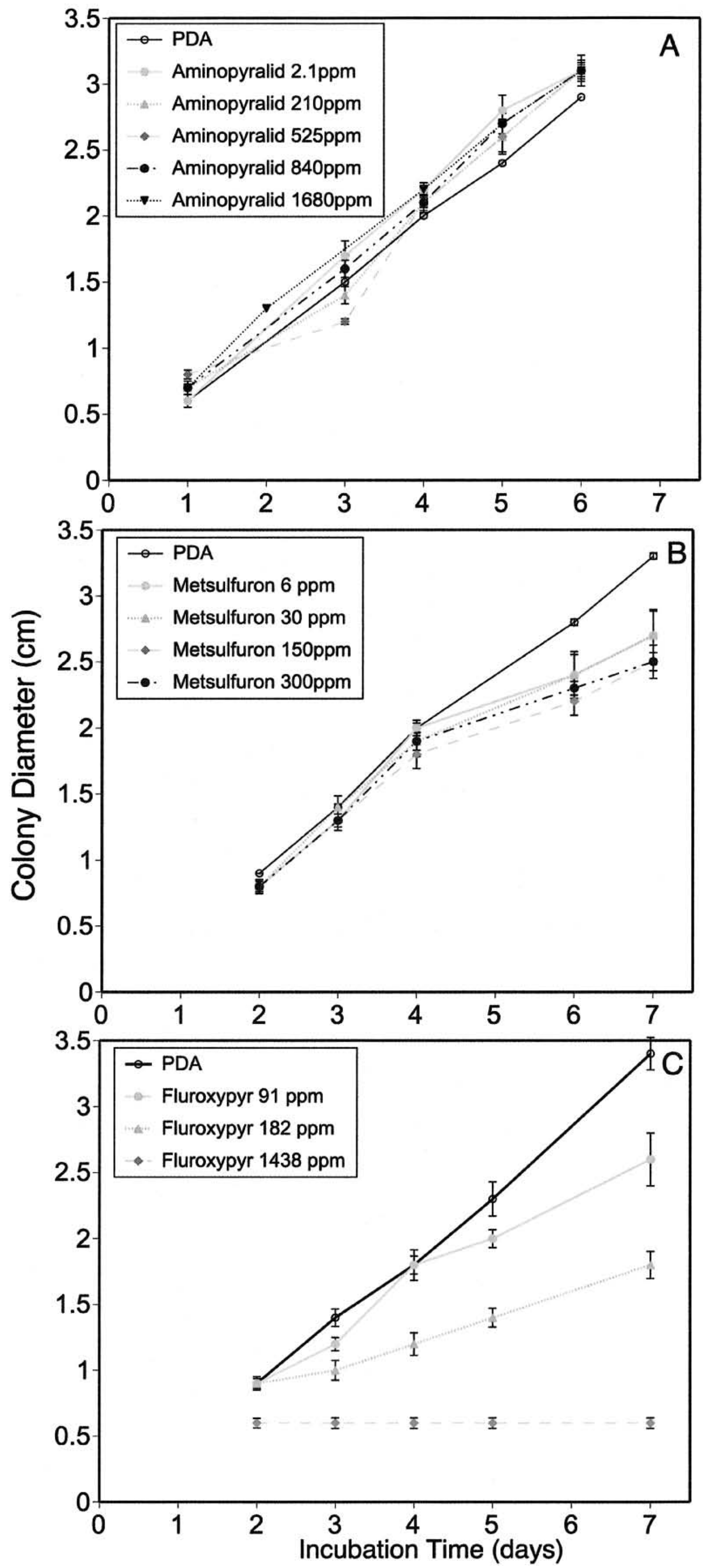

Figure 2. Growth inhibition of *M. verrucaria* by herbicides.

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