

Biological Control of Kudzu (*Pueraria lobata*) with an Isolate of *Myrothecium verrucaria*

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An isolate of the fungus *Myrothecium verrucaria* (MV) was evaluated for biocontrol potential against kudzu (*Pueraria lobata*). In greenhouse tests, MV was highly virulent against kudzu in the absence of dew when conidia were formulated in 0.2% Silwet L-77 surfactant (SW). Inoculum concentrations $\geq 2 \times 10^7$ conidia ml⁻¹ were required to satisfactorily control plants in the third leaf stage and larger. In controlled environment experiments, kudzu mortality was greater at higher temperatures (25-40°C) than at lower temperatures (10-20°C), although pathogenesis and mortality occurred at all temperatures tested. In field tests, transplanted kudzu seedlings in the 2-3 leaf growth stage treated with MV at 2×10^7 conidia ml⁻¹ in 0.2% SW, exhibited leaf and stem necrosis within 24 h following inoculation, with mortality occurring within 96 h. After 7 days, 100% of inoculated kudzu plants were killed in plots treated with the fungus/surfactant mixtures. Similar results were observed in a naturally occurring kudzu population, where 100% control occurred within 14 days after inoculation with 2×10^7 conidia ml⁻¹ in 0.2% SW. In summary, MV effectively controlled kudzu in the absence of dew over a wide range of physical and environmental conditions and under field conditions. These results indicate that, when properly formulated, MV has potential as a valuable bioherbicide for controlling kudzu.

Keywords: *Myrothecium verrucaria*, biological control, bioherbicide, kudzu, *Pueraria lobata*

INTRODUCTION

Kudzu [*Pueraria lobata* (Willd.) Ohwi] is a perennial leguminous vine native to eastern Asia. About fifteen species of *Pueraria* occur worldwide, but none are native to North or South America. Kudzu was introduced into the eastern and southern USA in the late 1800s (McKee & Stephens, 1943). In the early 1900s, kudzu was promoted as inexpensive forage in over-grazed pastures and for erosion control in the South (Piper, 1920). The US Soil Erosion Service provided over 80 million kudzu seedlings to southern homeowners for

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erosion control and land revitalization (Bailey, 1944). The federal government offered up to \$3.2 per ha as an incentive for farmers to plant kudzu, and by 1946, over 1.22 million ha had been planted. By the early 1950s, kudzu had spread throughout the southeastern USA and was no longer listed as a permissible cover plant under the Agricultural Conservation Program. By 1970, the US Department of Agriculture listed kudzu as a common weed in the South (Everest *et al.*, 1994), and in 1993 it was listed in a report by Congress as one of the most harmful nonindigenous plant species in the USA (United States Congress, OTA, 1993). In 1998, kudzu was added to the Federal Noxious Weed List by Congressional action, thus restricting its spread over state boundaries. At present, kudzu occurs in the USA from Florida to New York, westward to central Oklahoma and Texas, with the heaviest infestations in Alabama, Georgia and Mississippi (Miller, 1997).

Monetary losses from kudzu result mainly from reduced land productivity, primarily in forested areas. Losses in infested forests average \$19.4 ha⁻¹ year⁻¹ (Beckwith & Dangerfield, 1996). These losses in potential productivity have been estimated to be \$336 million each year for the 2.84 million infested hectares. Additional kudzu infestations have been estimated to be 48 600 ha year⁻¹, and thus, losses attributed to kudzu increase annually by nearly \$6 million. These estimates do not include costs for controlling the weed, which may amount to \$81 ha⁻¹ year⁻¹ (Miller, 1997). Kudzu also causes losses in biodiversity and reduced aesthetic values in recreational areas such as national parks, forests, and scenic highways in the southern and eastern USA.

Kudzu produces a large, starchy tuber from which annual regrowth occurs. Foliar sprays of picloram, picloram plus 2,4-D, dicamba plus 2,4-D, and tebuthiuron are effective after full leaf expansion occurs. Repeat sprays are necessary after regrowth appears. Herbicide treatments require yearly applications for up to 10 years for complete control. Burning and grazing may also be effective in some cases but are impractical in most heavily infested areas such as urban areas, and highway rights-of-way (Everest *et al.*, 1994).

Kudzu in the USA is represented by only one species (*P. lobata*) with apparently little genetic variability within the US population (Miller, 1997). Weed populations that exhibit limited genetic variability are considered as good candidates for biological control (Templeton & Trujillo, 1981).

Myrothecium verrucaria (Alb. & Schwein.) Ditmar:Fr. isolated from diseased sicklepod (*Senna obtusifolia* L.) exhibited excellent biocontrol potential for several weed species, including the legumes sicklepod and hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill], when formulated with the surfactant Silwet L-77 (a silicone-polyether copolymer spray adjuvant, OSi Specialties, Inc., Charlotte, NC, USA) in the absence of dew (Walker & Tilley, 1997). Kudzu was not examined as a potential host in this report and has not been previously reported as a host of *M. verrucaria*. In preliminary greenhouse tests, we found that the pathogen was highly virulent to kudzu in the absence of dew. The objectives of this study were to determine disease development and control of kudzu as influenced by inoculum concentration, plant growth stage, and post-inoculation temperature. In addition, bioherbicidal efficacy was examined under field conditions.

MATERIALS AND METHODS

Inoculum Production

Inocula (conidia) of *M. verrucaria* for all experiments were produced in Petri dishes containing potato dextrose agar (PDA; Difco Laboratories, Detroit, MI, USA)¹. The growth medium was inoculated by flooding each Petri dish with 3 ml of a suspension containing 2×10^6 conidia ml⁻¹. The inoculated plates were inverted and placed on open-mesh wire shelves of an incubator (Precision Scientific Inc., Chicago, IL 60647, USA) at 28°C for 5 days. Photoperiods (12 h) were provided by two 20 W cool-white fluorescent lamps positioned in the incubator door. Light intensity at dish level was approximately 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation as measured with a light meter. Conidia were rinsed from

the confluent lawns in each Petri dish culture with sterile, distilled water. The numbers of conidia were estimated with hemacytometers (approximately 2×10^9 conidia from each plate) and adjusted to the desired concentrations by adding distilled water.

Test Plant Propagation

Kudzu seed (obtained from Adams-Briscoe Seed Co., Jackson, GA 30233, USA) were placed on moistened filter paper contained in Petri dishes, and incubated at 28°C for 3 days in a darkened incubator to induce seed germination. Germinated seed were planted in 10 cm plastic pots (1 seed per pot) containing a 1:1 commercial potting mix (Jiffy Products of America, Inc., Batavia, IL 60510, USA)/sandy loam soil combination that was supplemented with a controlled-release (14:14:14, NPK) fertilizer (Grace Sierra Horticultural Products, Milpitas, CA 95035, USA) and placed on greenhouse benches. The plants were subirrigated daily. Temperatures in the greenhouse ranged from 28 to 32°C with 40–60% relative humidity (RH). The photoperiod was approximately 14 h, with 1600 to 1800 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation at midday.

Effect of Inoculum Concentration and Plant Growth Stage

Seedlings in either the cotyledonary, 1-3-leaf, 4-6-leaf, or 7-8-leaf growth stage were inoculated with either 0, 2×10^5 , 2×10^6 , 2×10^7 , or 2×10^8 conidia ml^{-1} suspended in 0.2% Silwet L-77 surfactant. Aerosol sprayers were used to apply the inoculum until the foliage was fully wetted. Following inoculation, the plants were placed directly on greenhouse benches and monitored for disease development. The data presented represent weed mortalities recorded fourteen days after inoculation. In this experiment and throughout, the treated plants were not subjected to a dew treatment. Greenhouse lighting and temperature conditions were as described previously. Experimental units consisted of groups of 10 pots, each containing one plant. The treatments were replicated three times and the experiment was conducted twice. In this experiment and throughout, mortality and dry weight data were transformed with the arc sin transformation prior to analysis. A randomized complete block experimental design was utilized, and data were analyzed using regression analysis and confidence limits (95% level).

Effect of Post-inoculation Temperature

Kudzu plants in the cotyledonary-to-first-true leaf stages of growth were inoculated by aerosol sprayers until the foliage was fully wetted with suspensions containing 2×10^7 conidia ml^{-1} plus 0.2% Silwet L-77 surfactant. Control plants were sprayed with 0.2% surfactant only. Experimental design was as described in the previous experiment. Immediately following inoculation the plants were placed in Shearer (Rheem Mfg. Co., Weaverville, NC 28787, USA) growth chambers at constant day/night temperatures of 10, 15, 20, 25, 30, 35 or 40°C. Photoperiods were 14 h day/10 h night with approximately 900 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation. Fourteen days after inoculation, all 10 plants of each experimental unit, both living and dead, were excised at the soil line, combined, and dried (80°C for 7 days) for dry-weight determinations. A randomized complete block experimental design was utilized. Data were analyzed using regression analysis and 95% confidence limits.

Field Experiments

Kudzu seedlings in the cotyledonary-to-first leaf growth stage were transplanted into 0.5 m² field microplots in separate experiments on June 27 and August 5, 1998. Each plot consisted of 10 seedlings spaced 30 cm apart. The plants were allowed to acclimate to field conditions for 1 week prior to treatment. Treatments consisted of 2×10^7 conidia ml^{-1} in distilled water, 2×10^7 conidia ml^{-1} in 0.2% Silwet L-77, distilled water only, and 0.2% Silwet L-77 only. The plants were sprayed until fully wetted (approximately 450 l ha^{-1}), resulting in inoculum densities of 9×10^{12} conidia ha^{-1} in those plots that received fungal treatments. Applications were made at midday with a hand-held pressurized sprayer. For the June 27

experiment, environmental conditions at the time of inoculation and for 24 h following treatment were: temperature at inoculation, 33°C with a RH of 56%. The high temperature for the 24 h period was 34°C, and the low temperature was 23°C. Maximum RH was 94%, with a light dew that lasted for a period of 3 h. For the August 5 experiment, environmental conditions at the time of inoculation and for 24 h following treatment were: temperature at inoculation, 34°C with a RH of 39%. The high temperature for the 24 h period was 36°C, and the low temperature was 24°C. Maximum relative humidity was 94%, with a light dew that lasted for a period of 3 h. The plants were monitored for disease development at 5-day intervals for 15 days, then harvested for dry weight determinations, as described previously for growth chamber experiments. The data presented represent weed mortalities recorded 15 days after inoculations. A randomized complete block design was utilized, and the treatments were replicated three times. Data from the June and August experiments were pooled following subjection to Bartlett's test for homogeneity (Steele & Torrey, 1980), and analyzed using analysis of variance.

A field test was conducted on July 19, 1998 in a site near Greenwood, MS that was heavily infested with a naturally occurring population of kudzu plants. Treatments consisted of: (1) 0.2×10^7 conidia ml^{-1} in distilled water; (2) 2×10^7 conidia ml^{-1} in distilled water; (3) 0.2×10^7 conidia ml^{-1} in 0.2% Silwet L-77 surfactant; (4) 2×10^7 conidia ml^{-1} in 0.2% Silwet L-77 surfactant; (5) 0.2% Silwet L-77 surfactant only; and (6) untreated controls. Spray volumes were applied at approximately 450 l ha^{-1} with backpack sprayers. This rate of spray resulted in inoculum densities of 9×10^{11} conidia ha^{-1} , and 9×10^{12} conidia ha^{-1} , respectively, for the low and high inoculum density rates. Plot sizes were 9.9 m^2 and treatments were replicated four times. Environmental conditions at the time of inoculation and for 24 h following treatment were: temperature at inoculation, 34°C with a RH of 50%. The high temperature for the 24 h period was 37°C, and the low temperature was 26°C. Maximum RH was 97%, with a light dew that lasted for a period of 5 h. Kudzu control was assessed visually by comparing the percentage of necrotic tissue in the treated plots with the tissue in the untreated plots. Estimates of control were made at weekly intervals for 4 weeks. The data presented represent weed mortalities recorded 28 days after inoculations. The test was arranged in a completely random design with three replications. Means were separated using Fisher's Protected Least Significant Difference (FLSD) at $P = 0.05$.

RESULTS AND DISCUSSION

Effect of Inoculum Concentration and Plant Growth Stage

There were significant increases in plant mortality at all growth stages as the inoculum density increased from 2×10^5 to 2×10^7 conidia ml^{-1} (Figure 1). The fungus applied at inoculum densities of 2×10^7 and 2×10^8 conidia ml^{-1} killed more plants when applied at lower densities. An inoculum density of 2×10^6 conidia ml^{-1} killed 40-50% of the weeds at the cotyledonary growth stage, but was less efficacious on older plants. Seedlings in the cotyledonary, 1-3-leaf, and 4-6 leaf stages were effectively killed with 2×10^7 conidia ml^{-1} , but plants in the 7-8-leaf stage required an inoculum concentration of 2×10^8 conidia ml^{-1} for 90% control (Figure 1).

Effect of Post-inoculation Temperature

Pathogenesis and mortality occurred at all temperatures that were tested; however, greater disease development and kudzu control occurred at higher temperatures (Figure 2). Disease symptoms were characterized by necrotic flecking which occurred within 6 h after inoculation at 30-40°C with slower disease development at lower temperatures (Figure 2). Disease symptoms progressed from inoculated cotyledons and leaves to produce stem lesions within 48 h after inoculation. These results indicate that this pathogen could be used in midsummer when similar temperatures occur in kudzu-infested regions of the southeastern USA.

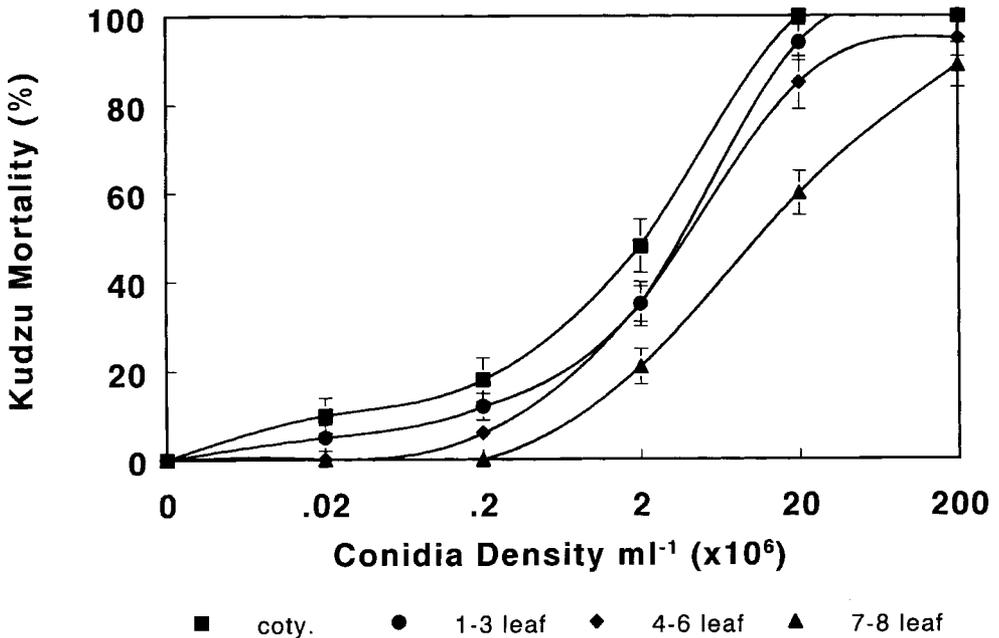


FIGURE 1. Effects of inoculum concentration and plant growth stage on biocontrol of kudzu (*Pueraria lobata*) by *Myrothecium verrucaria* in greenhouse experiments. Regression equations for the curves are: Cotyledonary, $y = 45.33 - 66.27x + 26.56x^2$, $r^2 = 0.96$; 1-3 leaves, $y = 47.67 - 67.16x + 24.71x^2$, $r^2 = 0.96$; 4-6 leaves, $y = 53.67 - 75.85x + 28.98x^2$, $r^2 = 0.94$; 5-7 leaves, $y = 27.67 - 36.49x + 11.11x^2$, $r^2 = 0.98$, where x represents plant growth stage (leaf number) and y represents weed mortality. Vertical bars represent 95% confidence intervals from two separate experiments, each consisting of three replications containing 10 plants per replication.

Field Experiments

In the microplot experiments, kudzu plants treated with the fungus/surfactant mixtures had developed leaf and stem necrosis within 24 h following inoculation, and were killed within 96 h. After 7 days, 100% of inoculated plants had been killed in plots treated with conidia/Silwet-L-77 suspensions (Table 1). No regrowth occurred on kudzu plants treated with the conidia/Silwet-L-77 suspensions during the 15 day time course of the experiments (data not shown). The fungus sporulated profusely on infected tissue and was easily reisolated. No visible damage or dry weight reductions were observed for kudzu plants in the untreated controls, for plants treated with surfactant only, or for plants treated with conidia suspended in distilled water only (Table 1).

Results observed in the natural kudzu infestation site were similar to the results obtained in the microplot experiments. Kudzu was completely controlled after 14 days in plots treated with 2×10^7 conidia ml⁻¹ and surfactant applied at 450 l ha⁻¹. Kudzu control was significantly less in plots treated with either the fungus in water only or the fungus in surfactant suspension at a rate of 2×10^6 conidia ml⁻¹ (Table 2). No regrowth occurred on vines that had been considered 'killed'; however, after 4 weeks, vines from the margins of untreated plots had begun to spread into treated areas where kudzu had been defoliated.

These results indicate that, when properly formulated, *M. verrucaria* can be a potential bioherbicide for controlling kudzu. Kudzu plants were effectively controlled in the absence of dew in greenhouse and growth chamber studies and under field conditions where temperatures approached or exceeded 40°C. The addition of Silwet L-77 to *M. verrucaria* conidial suspensions resulted in greatly increased mortality to kudzu, as was the case with

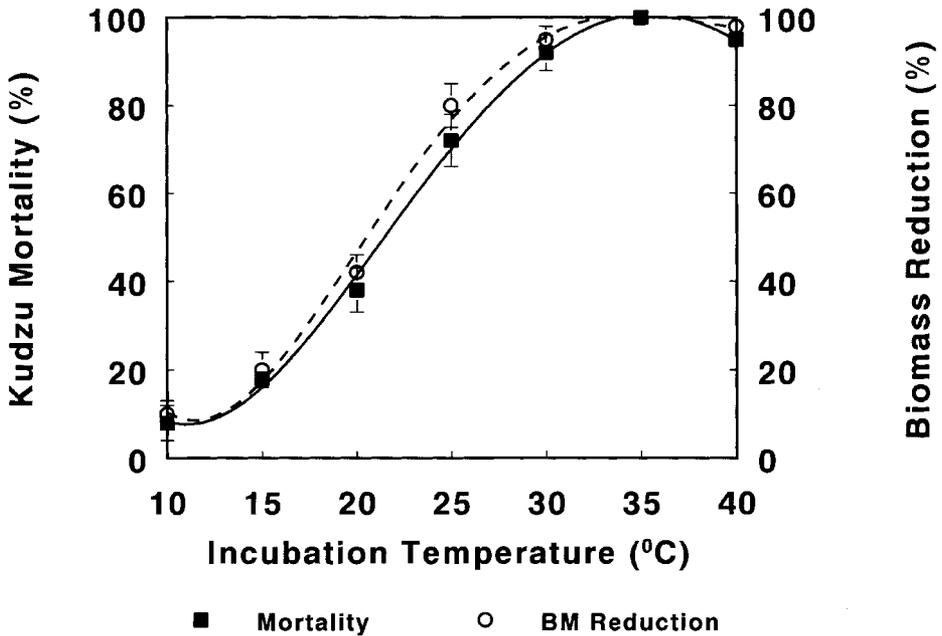


FIGURE 2. Effects of incubation temperature on mortality and biomass reduction of kudzu (*Pueraria lobata*) inoculated with *Myrothecium verrucaria* in growth chamber experiments. Regression equations for the curves are: Mortality; $y = 37 - 53.76x + 29.12x^2$, $r^2 = 0.96$, where x represents incubation temperature and y represents weed mortality; biomass reduction, $y = 56.43 - 84.67x + 45.79x^2$, $r^2 = 0.97$, where x represents incubation temperature and y represents biomass reduction. Vertical bars represent 95% confidence intervals. Means are from two separate experiments, each consisting of three replications containing 10 plants per replication.

TABLE 1. Biocontrol of kudzu (*Pueraria lobata*) with *Myrothecium verrucaria* in field microplots, Stoneville, MS, 1998

Treatment ^a	Mortality ^b (%)	Dry weight reduction (%)
Conidia + water ^c	0	0
Conidia + water + surfactant ^d	100	100
Water + surfactant ^c	0	0
Untreated	0	0

^aTreated plants were sprayed at a rate of 450 l ha⁻¹.

^bPlants were considered 'killed' when 100% necrosis occurred to individual plants.

^c 2×10^7 conidia ml⁻¹.

^d0.2% Silwet L-77 surfactant.

other weeds that have been previously tested (Walker & Tilley, 1997). Silwet L-77 has been reported to provide enhanced wetting of plant foliage and to increase stomatal infiltration of aqueous solutions (Field & Bishop, 1988; Zabkiewicz & Gaskin, 1989) in several different plant species. The extremely low oil-water surface tension (20 dynes cm⁻¹) created by Silwet L-77 has been shown to facilitate direct penetration by bacterial cells of *Pseudomonas syringae* pv. *phaseolicola* van Hall, into kudzu stomata, thereby enhancing infection of kudzu by this bacterial pathogen (Zidak *et al.*, 1992). A possible explanation for the increased mortality to kudzu and other susceptible hosts is that the relatively small size of

TABLE 2. Biocontrol of a natural infestation of kudzu (*Pueraria lobata*) with *Myrothecium verrucaria*, Greenwood, MS 1998

Treatment ^a	Mortality (%) ^b
Conidia (2×10^6 ml ⁻¹) + water	7
Conidia (2×10^7 ml ⁻¹) + water	20
Conidia (2×10^6 ml ⁻¹) + surfactant ^c	36
Conidia (2×10^7 ml ⁻¹) + surfactant ^c	100
Surfactant ^c only	5
Untreated	0
FLSD (0.05)	7

^aApplications were made with a backpack sprayer at a rate of 450 l ha⁻¹.

^bPlants were considered 'killed' when 100% necrosis occurred to individual plants.

^c0.2% Silwet L-77 surfactant in water.

M. verrucaria spores [$6.5\text{--}8.0 \times 2.0\text{--}3.5$ μm] (Domsch *et al.*, 1980) may allow for the direct penetration of conidia, phytotoxic fungal metabolites, or both, into stomata of potential host plants such as sicklepod and kudzu when the fungus is formulated with Silwet L-77. Previous research has shown that sicklepod stomatal openings are large enough to accommodate direct penetration by fungal spores that are in the size range of *M. verrucaria* spores (Van Dyke & Trigiano, 1987). More research is required to determine the infection processes.

Myrothecium verrucaria has been reported as a weak pathogen on several plant species (Nguyen *et al.*, 1973; Domsch *et al.*, 1980; Yang & Jong, 1995a). Virulence of other *M. verrucaria* isolates can be influenced by manipulation of inoculum concentration (Yang & Jong, 1995a). Because of the interaction of the surfactant with the high inoculum levels, secondary spread of the pathogen to uninoculated plants was not observed in our research or in other reports (Yang & Jong, 1995b). Sporodochial fluids and culture filtrates of *M. verrucaria* and other *Myrothecium* species have been reported to contain several non-specific phytotoxins (Pawar *et al.*, 1965; Cunfer & Lukezic, 1969; Jarvis *et al.*, 1985). In addition, some isolates of *M. verrucaria* have been reported to produce metabolites that are toxic to humans and livestock (Mortimer *et al.*, 1971; Domsch *et al.*, 1980; Jarvis *et al.*, 1985). We are conducting research to determine if the isolate used in our test produces potentially harmful substances, a critical point which should be addressed if this pathogen is to be used as a bioherbicide.

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NOTE

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

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