

Evaluation of the bioherbicide *Myrothecium verrucaria* for weed control in tomato (*Lycopersicon esculentum*)

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Abstract

An isolate of the fungus *Myrothecium verrucaria* was evaluated for its biocontrol potential against common purslane, horse purslane, spotted spurge, and prostrate spurge, all serious weed pests in commercial tomato fields in the southeastern US. In greenhouse and field tests, *M. verrucaria* was highly virulent against these weeds when applied as conidial sprays formulated in 0.2% Silwet L-77 surfactant, even in the absence of dew. In field test plots naturally infested with these weeds, seedlings in the two-to-three leaf growth stage treated with *M. verrucaria* at 2×10^7 conidia mL⁻¹ in 0.2% Silwet, exhibited leaf and stem necrosis within 24 h following inoculation, with mortality occurring within 96 h. After 7 days, *M. verrucaria* had killed 90–95% of both purslane species and 85–95% of both spurge species. Tomatoes that were transplanted into plots treated with *M. verrucaria* remained healthy and vigorous throughout the growing season. Since *M. verrucaria* effectively controlled several common weeds under field conditions, this fungus appears to have potential as an effective bioherbicide for pre-plant weed control in production systems with transplanted tomato.

Keywords: *Common purslane* (*Portulaca oleracea*), *horse purslane* (*P. portulacastrum*), *spotted spurge* (*Euphorbia maculata*), *prostrate spurge* (*Euphorbia prostrata*), *tomato* (*Lycopersicon esculentum*), *Myrothecium verrucaria*, *bioherbicide*, *biological weed control*, *surfactant*

Introduction

Common purslane (*Portulaca oleracea* L.), horse purslane (*P. portulacastrum* L.), spotted spurge (*Euphorbia maculata* L.), and prostrate spurge (*E. prostrata* Ait.) are weed pests in many areas of the southeastern US where tomatoes [*Lycopersicon esculentum* (Mill.) Swingle] are commercially grown (Bridges 1992). These weeds often form dense complexes and are difficult to control with chemical herbicides (Monks 1993).

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The bioherbicidal fungus *Myrothecium verrucaria* (Alb. & Schwein.) Ditmar has promise as a bioherbicide for controlling several divergent weed species, such as sicklepod [*Senna obtusifolia* (L.) Irwin & Barneby], hemp sesbania [*Sesbania exaltata* Rydb. ex. A.W. Hill] (Walker & Tilley 1997), and kudzu [*Pueraria lobata* (Willd.) Ohwi] (Boyette et al. 2002). Previous data on host range tests revealed that tomato plants inoculated with *M. verrucaria* were not killed, and exhibited only minor reductions in total biomass 2 weeks after inoculation (Walker & Tilley 1997). An effective biological control agent such as the strain of *M. verrucaria* used in these studies could offer an important weed control option to organically-grown tomatoes. Furthermore, tomato crops have the highest consumption of methyl bromide of all crop uses, accounting for 23% of pre-plant methyl bromide use. About 3773 tons are applied annually to the crop to control nematodes, insects and weeds. However, restrictions on usage and eventual EPA banning have resulted in searches for effective alternatives to methyl bromide (Roskopf et al. 2005). The use of effective bioherbicidal plant pathogens may offer such an alternative (Cook et al. 2005). The objectives of the present study were to evaluate the biocontrol efficacy of the fungus for controlling several weeds (i.e. common purslane, horse purslane, spotted spurge, and prostrate spurge) in tomato, and its effect on tomato growth.

Materials and methods

Chemicals

Potato dextrose agar (PDA) was purchased from Difco (Detroit, MI, USA). The surfactant Silwet L-77 was obtained from Osi Specialties, Inc. (Charlotte, NC, USA). The herbicide metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one], formulated as Sencor[®] (0.48 Kg a.i. L⁻¹), a product of Bayer Corp. (Kansas City, MO, USA), was purchased from a local supplier.

M. verrucaria culture and seed sources

An isolate of *M. verrucaria* (CMI Accession No. 368023) was used throughout these studies. A single strain of the fungus was used in all experiments. The fungus was preserved in screw-capped tubes containing sterilized soil (Bakerspigel 1953). Inocula (conidia) of *M. verrucaria* for all experiments were produced in Petri dishes containing PDA. The growth medium was inoculated by flooding each Petri dish with 3 mL of a suspension containing 2.0×10^6 conidia mL⁻¹. The inoculated plates were inverted and placed on open-mesh wire shelves of an incubator (Precision Scientific Inc., Chicago, IL, USA) at 28°C for 5 days. Twelve-hour photoperiods were provided by two 20W 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 (PAR) as measured with a light meter. After 5–7 days, conidia were rinsed from each Petri dish culture with sterile, distilled water. The concentrations of resulting suspensions were estimated using a haemocytometer and adjusted to 2.0×10^7 conidia mL⁻¹ by adding distilled water containing 0.2% Silwet L-77. Spurge and purslane seed were collected at the weed nursery of the Southern Weed Science Research Unit Stoneville, MS.

Greenhouse experiments

Seeds of *P. oleracea*, *P. portulacastrum*, *E. maculata* and *E. prostrata* were placed on the surfaces of moistened commercial soil mixtures (Jiffy Products of America, Inc., Batavia, IL, USA) contained in 10-cm diameter plastic pots, and then covered with a thin layer of the soil mixture. After the weed seedlings were in the first true-leaf growth stage (10–12 days old), the seedlings were thinned to two seedlings of each species pot⁻¹. A tomato (cv. 'Beefsteak') seedling (5–8 cm tall) was transplanted into each pot containing the weed seedlings. The pots were placed on greenhouse trays and allowed to grow an additional 14 days at 28°C. Plants were inoculated by spraying until the foliage was fully wetted (ca. 500 L ha⁻¹) with suspensions containing 2.0×10^7 conidia mL⁻¹ plus 0.2% Silwet. Control pots were sprayed with 0.2% Silwet only. Hand-held aerosol sprayers (Spra-Tool, AERVOE Industries, Gardnerville, NV, USA) were used to make all applications. New aerosol canisters were used to deliver high and equal pressure (and volume delivery) to all treatment sets. Each replicate contained ten pots, and each treatment was repeated three times. The experiment was repeated in time. Treatments consisted of: (1) a conidia:water suspension of *M. verrucaria*; (2) *M. verrucaria* conidia:0.2% Silwet; (3) 0.2% Silwet only; and (4) untreated. Following inoculations, the plants were placed on sub-irrigated greenhouse trays and visually monitored 14 days for disease development, and percentage of weed control was determined. Greenhouse temperatures were 28–32°C with 60–90% relative humidity. Day lengths were ca. 12 h with an average of 1850 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR at midday. Disease/damage was visually monitored and mortality recorded at the end of the tests. Surviving plants (weed and tomato seedlings) were excised at the soil line, oven-dried 48 h at 75°C, weighed, and percentages of biomass reductions were determined.

The experiments were repeated twice and data were averaged following subjection to Bartlett's test for homogeneity of variance (Gomez & Gomez 1984). A randomized complete block experimental design was used. Data were analyzed using ANOVA. Significant differences were determined at the 0.05 level of probability using Fisher's protected least significant difference (FLSD) at $P=0.05$.

Field experiments

Field test plots were established on 12 July 1998 and 9 September 1999 at the Jamie Whitten Delta States Research Center, Stoneville, MS, USA, on a site that was naturally infested with the purslane and spurge species described above. Grids were used to establish 1-m² plots. Average weed densities and plant heights were determined prior to treatment for field tests in 1998 (Table I). Similar weed densities and plant heights were treated in the 1999 field tests, and plant densities and heights were not significantly different (data not shown). Treatments consisted of: (1) a conidia:water suspension (2.0×10^7 conidia mL⁻¹); (2) conidia:0.2% Silwet (2.0×10^7 conidia mL⁻¹); (3) 0.2% Silwet only; (4) herbicide (metribuzin, 0.48 kg a.i. ha⁻¹) applied to established weed stands; and (5) untreated. Spray applications were made with hand-held sprayers at a carrier volume of 500 L ha⁻¹. Treatments were replicated four times. To determine possible residual effects of any of the above field treatments, tomato seedlings were transplanted at a density (one plant m⁻²) commonly used in commercial tomato production. Tomato (cv. 'Beefsteak') seedlings (10 cm tall) were transplanted 14 days after treatments were applied. Plant damage was visually

Table I. Average densities and plant heights of *E. maculata*, *E. prostrata*, *P. oleracea*, and *P. portulacastrum* in field plot experiments prior to treatment.¹

Weed species	Density (plants m ⁻¹)	Height (cm)
<i>E. maculata</i>	75	14
<i>E. prostrata</i>	67	7
<i>P. oleracea</i>	69	10
<i>P. portulacastrum</i>	37	17

¹Similar weed densities and plant heights were treated in the 1999 field tests, and plant densities and heights in each replication for the plots in both years were not significantly different.

monitored during the course of the experiments for 28 days after transplanting (data not shown), and mortality recorded at the end of the tests. Surviving plants (weed and tomato seedlings) were excised at the soil line, oven-dried for 48 h at 75°C, weighed, and percentages of biomass reductions were determined as compared to the untreated control plants.

Data were tested for homogeneity (Gomez & Gomez 1984) and pooled over the 2-year testing period and analyzed using the analysis of variance. Data were analyzed using ANOVA. Significant differences were determined at the 0.05 level of probability using FLSD.

Results and discussion

Greenhouse experiments

After 14 days, treatments with conidia in 0.2% Silwet under greenhouse conditions caused 94–98% mortality of each weed species, whereas treatments with conidia or Silwet alone were not significantly different from untreated controls (Table II). Disease symptomatology was characterized by necrotic flecking on leaves that coalesced into large lesions. Symptoms progressed, initially infecting cotyledons and leaves, and later (within 48 h) producing stem lesions. The fungus sporulated profusely on infected tissue and was easily reisolated. Although tomato seedlings were not visually affected by fungal treatments, a 10% reduction in dry weight of top

Table II. Effects of *M. verrucaria* on mortality of *E. maculata*, *E. prostrata*, *P. oleracea*, *P. portulacastrum*, and *L. esculentum* in greenhouse experiments, 14 days after treatment.

Treatment ¹	Plant species				
	<i>E. maculata</i>	<i>E. prostrata</i>	<i>P. oleracea</i>	<i>P. portulacastrum</i>	<i>L. esculentum</i>
	Mortality (%) ²				
Conidia in water	2 b	3 b	1 b	0 b	0 a
Silwet (0.2%)	0 b	0 b	0 b	0 b	0 a
Conidia:0.2% Silwet	94 a	96 a	95 a	98 a	0 a
Untreated	0 b	0 b	0 b	0 a	0 a

¹Conidial concentrations were 2.0×10^7 conidia mL⁻¹; Spray applications were made with hand-held aerosol sprayers; foliage was sprayed until fully wetted (ca. carrier volume of 500 L ha⁻¹). Treatments were replicated three times and the test was repeated in time. ²Means within a column followed by the same letter are not different according to Fisher's protected LSD test at $P=0.05$.

growth occurred with the conidia:Silwet treatment (Table III). Dew was not required to infect or kill these weeds, however, a suitable surfactant, e.g., Silwet was required to facilitate pathogenesis of *M. verrucaria* (Walker & Tilley 1997; Boyette et al. 2001, 2002). Although Silwet is effective, the mechanism(s) of its interaction with the pathogen and/or leaf surfaces are presently unknown, it may promote efficacy via increasing water retention, altering plant surfaces to enhance infection, and/or aid in the absorption of possible phytotoxic substances into plant tissues.

Field experiments

In field tests, treatments with conidia of *M. verrucaria* in 0.2% Silwet caused 90–95% mortality of both purslane species, and 80–85% of both spurge species 14 days after treatment (Table IV). The mortality effected by these treatments was equivalent to treatment with the herbicide metribuzin in the *Portulaca* species and only slightly less than the herbicide in the test species of spurge (Table IV). Similarly, the dry weight reductions of top growth caused by these treatments (Table V) reflected the weed mortality effects (Table IV). Because tomato seedlings were not susceptible to the bioherbicide or surfactant treatments in the greenhouse (Tables II and III), and because commercial tomato culture utilizes weed ‘burn-down’ prior to transplantation (Peet 2000), tomato seedlings were not transplanted in our field tests until 14 days after treatment applications. Transplanted tomato seedlings in these plots treated with *M. verrucaria*:Silwet remained healthy and vigorous, and exhibited no evidence of disease or injury throughout the growing season (data not shown). All treatments in these greenhouse and field tests were performed without dew, which for most bioherbicides is often one of the most critically needed factors for biological and economical practicality (Boyette et al. 1996; Weidemann et al. 1996).

Results presented in our studies have shown that dew is not required by *M. verrucaria* to control these spurge and purslane species in the greenhouse or in the field. Previous attempts were made to develop another fungus, *Dichotomophthora portulacae* Mehrlich & Fitzpatrick ex M.B. Ellis, as a biological control agent for common purslane in the state of New York, USA, but tests were discontinued due to inadequate humidity and dew requirements needed for plant infection during the production season (Klisiewicz 1985). In those greenhouse studies, *D. portulacae*

Table III. Effects of *M. verrucaria* on dry weight accumulation of *E. maculata*, *E. prostrata*, *P. oleracea*, *P. portulacastrum*, and *L. esculentum* in greenhouse experiments, 14 days after treatment.

Treatment ¹	Plant species				
	<i>E. maculata</i>	<i>E. prostrata</i>	<i>P. oleracea</i>	<i>P. portulacastrum</i>	<i>L. esculentum</i>
	Dry weight (% reduction) ²				
Conidia in water	2 b	3 b	1 b	0 b	0 b
Silwet	2 b	3 b	1 b	3 b	2 b
Conidia:Silwet	94 a	96 a	95 a	98 a	10 a
Untreated	0 b	0 b	0 b	0 a	0 b

¹Conidial concentrations were 2.0×10^7 conidia mL⁻¹; Silwet concentrations were 0.2%. Spray applications were made with hand-held aerosol sprayers; foliage was sprayed until fully wetted (ca. carrier volume of 500 L ha⁻¹). Treatments were replicated three times and the test was repeated in time. ²Means within a column followed by the same letter are not different according to Fisher's protected LSD test at $P=0.05$.

Table IV. Effects of *M. verrucaria* on mortality of *E. maculata*, *E. prostrata*, *P. oleracea*, *P. portulacastrum*, and *L. esculentum* in field experiments, 14 days after treatment.

Treatment ¹	Plant species				
	<i>E. maculata</i>	<i>E. prostrata</i>	<i>P. oleracea</i>	<i>P. portulacastrum</i>	<i>L. esculentum</i>
	Mortality (%) ²				
Conidia in water	2 c	3 b	1 b	0 b	0 a
Silwet	0 c	0 b	0 b	0 b	0 a
Conidia:Silwet	80 b	85 a	90 a	95 a	0 a
Metribuzin	93 a	92 a	95 a	95 a	0 a
Untreated	0 c	0 b	0 b	0 a	0 a

¹Conidial concentrations were 2.0×10^7 conidia mL⁻¹; Silwet concentrations were 0.2%; metribuzin rates were 0.48 kg a.i. ha⁻¹. Spray applications were made with hand-held sprayers at a carrier volume of 500 L ha⁻¹. Treatments were replicated four times. ²Means within a column followed by the same letter are not different according to Fisher's protected LSD test at $P=0.05$.

caused infection of weeds at temperatures ranging from 15–33°C, with symptoms developing within 48 h after inoculation (Klisiewicz 1985). Similarly, the fungus *Gibbago trianthemae* Simmons was evaluated as a bioherbicide for horse purslane control, but lengthy free moisture requirements (up to 3 days) also limit its practical usage (Mitchell 1988; Aneja & Kaushal 1999; Aneja et al. 2000).

Other strains of *M. verrucaria* have also been evaluated as biocontrol agents. For example a strain that was originally isolated from an exotic invasive rangeland weed, leafy spurge (*Euphorbia esula* L.) was evaluated as bioherbicide for leafy spurge (Yang & Jong 1995a,b), and morningglory (*Ipomoea*) species in sugarcane [*Saccharum officinarum* L.] (Milhollon et al. 2003). A different strain, isolated from Faba bean (*Vicia faba* L.) showed promise as a bioherbicide for *Orobanche crenata* Forsk in Faba bean. (El-Kassas et al. 2004). These strains appear to be pathologically distinct from each other as well as from the *M. verrucaria* isolate used in our studies. The *M. verrucaria* strain used in the results reported herein has been shown to exhibit a broad host range (Walker & Tilley 1997; Anderson & Hallett 2004). However, this is the first report of this strain of *M. verrucaria* being evaluated against spurge and

Table V. Effects of *M. verrucaria* on dry weight reduction of *E. maculata*, *E. prostrata*, *P. oleracea*, *P. portulacastrum*, and *L. esculentum* in field experiments, 14 days after treatment.

Treatment ¹	Plant species				
	<i>E. maculata</i>	<i>E. prostrata</i>	<i>P. oleracea</i>	<i>P. portulacastrum</i>	<i>L. esculentum</i>
	Dry weight (% Reduction) ²				
Conidia in water	2 b	3 b	1 b	0 b	0 a
Silwet	2 b	3 b	1 b	2 b	0 a
Conidia:Silwet	96 a	96 a	97 a	97 a	0 a
Metribuzin	96 a	94 a	95 a	96 a	0 a
Untreated	0 b	0 b	0 b	0 b	0 a

¹Conidial concentrations were 2.0×10^7 conidia mL⁻¹; Silwet concentrations were 0.2%; metribuzin rates were 0.48 kg a.i. ha⁻¹. Spray applications were made with hand-held sprayers at a carrier volume of 500 L ha⁻¹. Treatments were replicated four times. ²Means within a column followed by the same letter are not different according to Fisher's protected LSD test at $P=0.05$.

purslane species. Our results suggest that *M. verrucaria* has potential for control in tomato. The critical weed-free period for tomatoes is about 4–5 weeks after transplanting (Monks 1993). We have shown that it is possible to control spurge and purslane weeds with *M. verrucaria* during this period when weed competition must be suppressed to avoid yield reductions. In addition to the potential for use of this pathogen as a methyl bromide alternative, in commercially-grown tomato, since tomatoes are an important commodity in organic farming systems which restrict the use of synthetic pesticides, a bioherbicide such as *M. verrucaria* may have utility.

As a bioherbicide, *M. verrucaria* also has potential to control several invasive weeds such as sicklepod and hemp sesbania (Walker & Tilley 1997); redivine, trumpet creeper (Boyette et al. 2001, 2007 (in press)); and kudzu (Boyette et al. 2002). Since kudzu is a major problem on low-value, non-agronomic lands, and traditional weed control options have not been well established, it may be an attractive target for commercial bioherbicide development.

One concern however, is that many fungi, including some species of *Myrothecium*, produce a class of mycotoxins called trichothecenes (e.g., the roridins and verrucarins) (Jarvis et al. 1985). Trichothecenes represent a large class of secondary metabolites that cause a wide range of acute and chronic effects in mammals (D'Mello et al. 1999). Their presence has hindered EPA registration of *M. verrucaria* isolate (CMI Accession Number 368023) as a bioherbicide, although heat-killed cells of another *M. verrucaria* strain have been registered as a nematocide (Warrior et al. 1999). Other studies have indicated that trichothecenes were not detected in infected sicklepod and kudzu plants inoculated with MV (Abbas et al. 2001). Since this strain produces these undesirable compounds (Abbas et al. 2001), our future research will attempt to eliminate or reduce their levels using inhibitors, mutant selection, and cultural methods (Hoagland et al. 2005, 2007 (in press)). If we are successful, *M. verrucaria* could become a valuable bioherbicide that could control several economically important weeds.

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