Host Range Determination of *Myrothecium verrucaria* Isolated from Leafy Spurge

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ABSTRACT

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The host range of *Myrothecium verrucaria* (ATCC 90310) isolated from leafy spurge (*Euphorbia esula*) collected in China was determined to be 54 plant species in 42 genera and 11 families following artificial inoculation. The number of plant species infected was influenced by the dew temperature or invert emulsion (IE, water-in-oil) carrier for the inoculum. Of 46 plant species inoculated with an aqueous conidial suspension and incubated for 18 h in dew chambers at 15, 20, 25, and 30°C, 1, 6, 15, and 39 plant species, respectively, developed disease. Velvetleaf (*Abutilon theophrasti*) was killed by this pathogen only when the plants were inoculated with IE carrier plus conidia, but not when atomized with an aqueous conidial suspension and incubated in dew chambers at 30°C for 18 h. Disease severity on thistle (*Carduus* spp.) and starthistle (*Centaurea* sp.) was greatly affected by the use of the IE carrier; high inoculum levels of *M. verrucaria* were needed to kill older thistle plants. *Myrothecium verrucaria* has potential as a mycoherbicide against annual weeds.

Myrothecium verrucaria (Albertini & Schwein.) Ditmar:Fr., which has been isolated from soil, air, and plant materials (6), is a facultative parasite of numerous plants, including peanut (*Arachis hypogaea* L.) (1), cucumber (*Cucumis sativus* L.) (7,10), soybean (*Glycine max* (L.) Merr.) (9), upland cotton (*Gossypium hirsutum* L.) (2,7,8), sunflower (*Helianthus annuus* L.) (5), birdsfoot trefoil (*Lotus corniculatus* L.) (3,7), tomato (*Lycopersicon esculentum* Mill.) (12,14), alfalfa (*Medicago sativa* L.) (4), rice (*Oryza sativa* L.) (9,11), red clover (*Trifolium pratense* L.) (4), and corn (*Zea mays* L.) (7). Yang and Jong (18) also found that *M. verrucaria* was pathogenic to nine species of *Euphorbia* (*E. corollata* L., *E. cyathophora* Murr., *E. cyparissias* L., *E. esula* L., *E. helioscopia* L., *E. heterophylla* L., *E. lathyrus* L., *E. marginata* Pursh. *varigata*, *E. virgata* Waldst. & Kit.), and reed canarygrass (*Phalaris arundinacea* L.).

An isolate of *M. verrucaria*, ATCC 90310, obtained from leafy spurge (*E. esula*) collected in China in 1990 (15,19), has not been tested for pathogenicity on other hosts and the effect of dew temperatures on infection of other weed plant species by this isolate has not been determined.

Myrothecium verrucaria, like *Alternaria alternata* (Fries) Keissler and *A. angustiovoidea* Simmons, can kill leafy spurge in the absence of dew when the inoculum is suspended in a carrier (an invert emulsion [IE]) and sprayed onto the plants (17,18). However, the IE is difficult to spray when the ambient temperature is at or below 25°C (S.-M. Yang, *unpublished data*). An IE could enhance the infection of weed plants by isolate ATCC 90310 in the absence of dew. The effect of different ages or growth stages of weeds on the efficacy of isolate ATCC 90310 of *M. verrucaria* on weeds is not known.

The objectives of this study were to determine (i) the host range of isolate ATCC 90310, (ii) the effect of different dew temperatures on infection of plant species, (iii) the effect of improved IE on infection of weeds, and (iv) the effective dosages of inoculum required to kill different ages of plants in the absence of dew.

MATERIALS AND METHODS

Plant materials. Plants used in this study were grown in clay pots (10 cm diameter) or plastic pots (10 × 10 cm) from seeds, except leafy spurge, sweet potato (*Ipomoea batatas* (L.) Lam.) and potato (*Solanum tuberosum* L.). Leafy spurge (FD7, Bozeman, Mont.) and sweet potato were propagated from root pieces, and potato from tubers. A pasteurized greenhouse soil mix consisting of sand, limestone, fertilizer (10-10-10), Aqua Grow "G" granular, and vermiculite was placed in pots before planting (16).

Preparation of inoculum. The isolate of *M. verrucaria* (ATCC 90310) used in this study was obtained from leafy spurge collected in Dongsheng, Inner Mongolia, China, in 1990 as previously described (18). Single-spore cultures of this isolate have been stored in liquid nitrogen since 1990. The fungus was grown on potato-dextrose agar (PDA) with antibiotics (penicillin G [30 mg per liter] and streptomycin sulfate [100 mg per liter]) for 4 to 6 weeks at 30°C. Aqueous stock conidial suspensions were prepared by suspending the conidia in 2% sucrose and 0.1% Tween 20 (ST) solution, and filtering through two layers of cheesecloth prior to inoculation. The final conidial concentration was adjusted to 1×10^8 per ml in ST solution using a hemacytometer.

An improved IE (water-in-oil type) that could be used at or above 25°C was tested to aid infection of test plant species in the absence of dew. The IE was prepared by mixing the ST solution with an oil phase (1:1, vol/vol). For inoculum, conidia suspended in ST solution adjusted to 2×10^8 conidia per ml, as above, were mixed with the oil phase. The final conidial concentration used for inoculation was 1×10^8 conidia per ml.

Inoculation methods. Two procedures were used to inoculate the plants. First, plants were atomized (70×10^2 kg per m^2 = 10 lb/in^2) with the aqueous conidial suspension from 20 cm away until run-off, following which they were immediately incubated in dew chambers at 30°C for 18 h, then moved to greenhouse benches until disease severity was rated. Control plants were atomized with ST solution only. Second, plants were sprayed with 100 ml, unless otherwise stated, of IE only (control) or IE plus conidia onto 25 or 30 pots in a 1-m^2 area (= 10^3 liters per ha = 106 gallons per acre) with a 3.785-liter (1 gallon) garden sprayer with a T-Jet 8002 nozzle (Spraying System Co., Wheaton, Ill.) as described previously (17). After spraying, the plants were directly placed on greenhouse benches until disease severity was rated. All experiments were repeated once. The greenhouse temperature range was 22 to 34°C and relative humidity range was 33 to 64%. For each inoculation test, five pots (10 cm diameter clay pots or 10 × 10 cm plastic pots) were used for each plant species. Each pot contained one seed-propagated plant, 5 to 10 grass tillers, and 1 to 5 shoots for root- or tuber-

Names are necessary to report factually on available data, however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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propagated plants. All plants used were 3 to 4 weeks old after planting.

Disease rating. Disease severity was rated 2 weeks after inoculation using an arbitrary 1 to 4 disease index (DI) rating scale to indicate severity: 0 = no infection or macroscopic lesions on leaves; 1 = tip of leaves curled, brown, or less than 10 spots on a leaf; 2 = more than 10 spots on a leaf, spots coalesced, some leaves yellow or brown, or lower leaves dropped but plant top still green; 3 = plant top brown or killed with leaves brown or dropped but stems green, or for grasses, more than 50% of the leaves dead; 4 = whole plants dying or dead. The average disease severity rating was calculated from the two repetitions and presented as a minus (–) or plus (+): – = no infection (DI = 0); + = slight to moderate infection (DI = 0.1 to 2.9, resistant); ++ = severe infection (DI =3.0 to 3.9, susceptible); and +++ = plants dead (DI = 4.0, very susceptible). After disease severity was recorded, infected plants were randomly selected for reisolation of the pathogen. Tissue pieces (0.3 × 0.8 cm) were cut from lesions or the dead portions of severely infected plants, surface disinfected in 50% Clorox, and washed in sterile distilled water for 5 min. The tissue pieces were then placed on antibiotic PDA plates and incubated at 30°C for 2 weeks. Formation of sporodochia with conidia typical for *M. verrucaria* was used to positively identify the pathogen (6).

Host range. Sixty-two plant species in 48 genera and 11 families were atomized with ST solution (control) and aqueous conidial suspensions and incubated in a dew chamber at 30°C for 18 h and then moved to the greenhouse as previously described. Each plant species had five pots and the test was repeated once as previously described.

Effect of dew temperature on infection. Forty-six plant species were inoculated with ST (control) or aqueous conidial suspensions of *M. verrucaria* and incubated in dew chambers at four different temperatures (15, 20, 25, and 30°C) for 18 h. Each plant species had five pots and the test was repeated once as previously described.

Effect of IE on infection of weed plant species in the absence of dew. Seven weed species, (5 pots per species, 35 pots total in a meter square area) were selected and sprayed using the 1-gallon garden sprayer with 100 ml of ST only, aqueous conidial suspension, IE carrier only, or IE carrier plus conidia of *M. verrucaria,* and directly placed on the greenhouse benches as previously described. Additional plants inoculated with aqueous conidial suspensions were placed in the dew chamber at 30°C for 18 h before being moved to the greenhouse. The test was repeated once.

Effect of plant age and inoculum dose. Twenty-five or 30 pots of different ages (5 pots per species per age, two- to thirty-three-true-leaf stages) of 2 or 3 plant species were inoculated with 100 ml, 200 ml, and 400 ml of IE, or IE plus conidia. The test was repeated once.

RESULTS

Host range. *Myrothecium verrucaria* infected 54 of the 62 plant species tested. Among these, 42 plant species in 31 genera and 8 families were new hosts of this fungus. Of the 54 infected plant species, 27 were susceptible and are as follows: (common names followed by reference[s] for those hosts previously reported): *Amaranthus retroflexus* L. (redroot pigweed) (*Amaranthaceae*); *Carduus pycnocephalus* L. (Italian thistle), *Carthamus tinctorius* L. cv. Catin 13 (safflower), sunflower cv. TT894 (5) (*Asteraceae*); *Chenopodium album* L. (common lamb's-quarters), *C. quinoa* Willd. (quinoa), *Spinacia oleracea* L. cv. Bloomsdale Long Standing (spinach) (*Chenopodiaceae*); *Convolvulus arvensis* L. (field bindweed), *Ipomoea purpurea* (L.) Roth. (tall morning-glory) (*Convolvulaceae*); cucumber cv. Straight Eight (7,10) (*Cucurbitaceae*); leafy spurge FD#7 (15,18) (*Euphorbiaceae*); *Cicer arietinum* L. cv. Ethiopia (chickpea), soybean cv. Williams (7,9), alfalfa cv. Williamsburg (4), *Pisum sativum* L. cv. Puget (pea), red clover (4), *Vicia sativa* L. (common vetch) (*Fabaceae*); cotton cv. E-Z Red (2,7,8) (*Malvaceae*); *Agrostis gigantea* Roth (redtop), *Bouteloua gracilis* (H. B. K.) Lag. ex Steud. (blue grama), *Eragrostis trichodes* (Nutt.) A. Wood (sand lovegrass), reed canarygrass (18), corn cv. DK 677 (7) (*Poaceae*); *Polygonum convolvulus* L. (wild buckwheat), *Rumex crispus* L. (curly rock) (*Polygonaceae*); *Capsicum annuum* L. cv. North Star (pepper) and *Solanum tuberosum* L. (potato) (*Solanaceae*). Although *M. verrucaria* infected the four grasses and aboveground stems of leafy spurge and potato, new tillers and shoots or stems subsequently produced from the basal stems, underground root buds, or tubers, were not affected by the pathogen.

The remaining 27 species were slightly infected as follows: *Carduus acanthoides* L. (plumeless thistle), *C. tenuiflorus* Curt. (slenderflower thistle), *C. thoermeri* Weinm. (musk thistle), *Centaurea calcitrapa* L. (purple starthistle), *C. diffusa* Lam. (diffuse knapweed), *C. maculosa* Lam. (spotted knapweed), *C. solstitialis* L. (yellow starthistle), *Chondrilla juncea* L. (skeletonweed), *Cirsium arvense* (L.) Scop. (Canada thistle) (*Asteraceae*); *Ipomoea aquatica* Forssk. (water-spinach), *I. batatas* (L.) Lam. (sweet potato), *I. hederacea* (L.) Jacq. (ivyleaf morning-glory) (*Convolvulaceae*); peanut cv. Wilco (1), *Medicago lupulina* L. (black medic), *Phaseolus vulgaris* L. cv. Black Valentine (bean) (*Fabaceae*); *Andropogon hallii* Hack. (Sand bluestem), *A. scoparius* Michx. (little bluestem), *Bromus inermis* Leyss. (smooth brome), *Calamovilfa longifolia* (Hook.) Lams.-Scribn. (prairie sandreed), *Dactylis glomerata* L. (orchardgrass), *Festuca* sp. (fescue), rice cv. M201 (9,11), *Panicum virgatum* L. (switchgrass), *Phleum pratense* L. (timothy grass), *Sorghastrum nutans* (L.) Nash (Indian grass), *Triticum aestivum* L. cv. Max (wheat) (*Poaceae*); and tomato cv. Red Rock (12,14) (*Solanaceae*).

Plants in the same genus, e.g. *Carduus, Centaurea,* and *Chenopodium,* reacted similarly to *M. verrucaria* except for Italian thistle, which was very susceptible to *M. verrucaria* while other thistles were resistant. However, plants in the genus *Ipomoea* reacted differently to *M. verrucaria:* ivyleaf morning-glory, sweet potato, and water spinach were resistant but tall morning-glory was susceptible.

Most plant cultivars reacted uniformly to *M. verrucaria.* Three cultivars of chick-

Table 1. Effect of 18-h dew chamber treatment at various temperatures on infection of different plant species by *Myrothecium verrucaria*[a]

Plant species	Dew chamber temperatures (C)			
	15	20	25	30
Carduus acanthoides L.	–[b]	–	–	+
Amaranthus retroflexus L.	–	–	–	++
Rumex crispus L.	–	–	–	+++
Ipomoea aquatica L.	–	–	–	+
Polygonum convolvulus L.	–	–	+	++
I. purpurea (L.) Roth.	–	–	++	++
Chenopodium album L.	–	–	++	+++
Vicia sativa L.	–	+	++	++
Convolvulus arvensis L.	–	+	+	+++
Euphorbia esula L.	–	+	+	+++
C. pycnocephalus L.	–	++	+++	+++
Carthamus tinctorius L.	++	++	++	+++

[a] Plants 3 to 4 weeks old were used. Conidial concentration was 1.0×10^8 conidia per ml. Following inoculation, plants were incubated in dew chambers at different temperatures for 18 h and then moved to greenhouse benches (22 to 34°C, 33 to 60% relative humidity) for another 13 days, after which disease severity was rated.

[b] – indicates no infection (disease index [DI] = 0); + indicates slight infection (DI = 0.1 to 2.9); ++ indicates some plants were severely infected and some dead (DI = 3.0 to 3.9); and +++ indicates all plants dead (DI = 4.0). See text for DI determination.

pea (Ethiopia, India, and USA), all five sunflower inbred lines (HA R1 to R5) of North Dakota State University and one cultivar (TT894) of Texas Triumph (Lubbock, Tex.), and the three safflower cultivars (Pacific 1, UC 41, and Catin 13) were susceptible to *M. verrucaria*, while five cultivars (Celebrity, Florida, Heinze, Sunny, and Red Rock) of tomato were not.

None of the control plants sprayed with sucrose solution (control, not shown in Table 1) were damaged or symptomatic. *Myrothecium verrucaria* was frequently reisolated from tissue pieces of randomly selected severely infected plants.

The eight plant species not infected by *M. verrucaria* were *Chromolaena odorata* (L.) R. M. King & H. Robinson (Siam weed), *Lactuca sativa* L.(lettuce) (*Asteraceae*); *Cucumis melo* L. (cantaloupe) (*Cucurbitaceae*); *Trifolium repens* L. (white clover) (*Fabaceae*); *Abelmoschus esculentus* (L.) Moench cv. Cleamson Spineless (okra), *Abutilon theophrasti* Medik. (velvetleaf) (*Malvaceae*), *Rottboellia cochinchinensis* (Lour.) W. Clayton (itchgrass) (*Poaceae*), and *Physalis ixocarpa* Brot. ex Hornem (Mexican groundcherry) (*Solanaceae*).

Effect of dew temperature on infection. Dew temperatures affected the amount of disease on plant species inoculated with *M. verrucaria*. Of the 46 plant species, 1, 6, 15, and 39 developed disease at 15, 20, 25, and 30°C dew temperatures, respectively. Sunflower and safflower also were infected after 72-h incubation at dew temperatures of 20 and 15°C, respectively. The optimum dew temperature for disease development is 30°C.

Among the 39 plant species that had been incubated in dew chambers at 30°C, 24 species (redroot pigweed, spinach, cucumber, soybean, pea, red clover, cotton, corn, potato, curly dock, pepper, plumeless thistle, slenderflower thistle, musk thistle, purple starthistle, diffuse knapweed, spotted knapweed, yellow starthistle, skeletonweed, Canada thistle, sweet potato, water spinach, black medic, and wheat) developed disease only at the highest dew temperature (30°C); 9 species (common lamb's-quarters, tall morning-glory, chickpea, alfalfa, wild buckwheat, peanut, bean, rice, and tomato) at two dew temperatures (30 and 25°C); 5 species (Italian thistle, common vetch, sunflower, field bindweed, and leafy spurge) at three dew temperatures (30, 25, and 20°C), and only 1 species (safflower) was severely infected at all four dew temperatures. The effect of dew temperatures on infection of 12 of the 39 plant species by *M. verrucaria* is shown in Table 1.

Dew temperature also affected disease severity on some plant species inoculated with *M. verrucaria*. Redroot pigweed, spinach, cucumber, soybean, pea, red clover, cotton, corn, potato, curly dock, and pepper were severely infected or killed at 30°C dew temperature, but no infection occurred at 25°C or at lower dew temperatures. Alfalfa, wild buckwheat, leafy spurge, field bindweed, and sunflower were severely infected or killed at 30°C dew temperature, but only slightly infected at 25°C or lower dew temperatures.

The eight plant species (Siam weed, lettuce, cantaloupe, white clover, okra, velvetleaf, itchgrass, and Mexican groundcherry), not infected by *M. verrucaria* at 30°C, were also not infected at the three lower dew temperatures.

Effect of IE on infection of weed plant species in the absence of dew. *Myrothecium verrucaria* severely infected or killed the seven weeds in the absence of dew using the improved IE carrier although the pathogen infected water spinach and four thistles slightly but not velvetleaf when the plants were inoculated with aqueous conidial suspensions and incubated in a dew chamber at 30°C for 18 h (Table 2). The pathogen caused no infection on any plant species when plants were inoculated with aqueous conidial suspension and immediately kept in the greenhouse in the absence of dew. Although the pathogen severely infected Canada thistle, the thistle rapidly produced new shoots from the underground roots. Repeated inoculations of the new shoots with the pathogen eventually killed the plants. *Myrothecium verrucaria* was reisolated from tissue pieces of randomly selected infected plants. Some of plants sprayed with IE alone were slightly injured, but no *M. verrucaria* was isolated.

The improved IE carrier was easily ap-

Table 2. Effect of invert emulsion (IE) on infection of seven weeds by *Myrothecium verrucaria* in the greenhouse[a]

Plant species	ST solution	Conidial suspension		IE	
		without dew	with dew	without conidia	with conidia
Abutilon theophrasti	–[b]	–	–	+	++
Cirsium arvense	–	–	+	–	++
Ipomoea aquatica	–	–	+	–	++
I. hederacea (L.) Jacq.	–	–	+	–	++
Carduus acanthoides	–	–	+	+	+++
C. tenuiflorus	–	–	+	–	+++
Centaurea calcitrapa	–	–	+	–	+++

[a] 35 pots (5 pots per species) in a 1-m^2 area were inoculated with 100 ml of sucrose–Tween 20 (ST) solution, conidial suspension, IE only, or IE plus conidia of *M. verrucaria* without dew in the greenhouse (25 to 32°C, 35 to 64% relative humidity). Plants inoculated with conidial suspension with dew indicates that the inoculated plants were incubated in a dew chamber at 30°C for 18 h after inoculation. Final conidial concentration for inoculation was 1.0×10^8 conidia per ml.

[b] – indicates no infection (disease index [DI] = 0), + indicates slight infection or injury (DI = 0.1 to 2.9); ++ indicates some plants were severely infected and some were dead (DI 3.0 to 3.9); and +++ indicates all plants dead (DI = 4.0). See text for DI determination.

Table 3. Effective dosages of *Myrothecium verrucaria* required to kill different growth stages of weeds using invert elusion (IE) in absence of dew in the greenhouse[a]

Plant species	Plant age (weeks)	IE only	Amount of IE + *M. verrucaria*		
			100 ml per m^2	200 ml per m^2	400 ml per m^2
Amaranthus retroflexus	2	–	+++[b]	+++	+++
	4,6	–	++	+++	+++
Carduus acanthoides	2	–	+++	+++	+++
	4,6	–	++	+++	+++
	9	+	++	++	+++
Carduus pycnocephalus	2,4	–	+++	+++	+++
	6	–	++	+++	+++
	8	+	+	+++	+++
Carduus tenuiflorus	2,5	–	+++	+++	+++
	9	–	+	++	+
Chenopodium album	2,4,6	+	+++	+++	+++
	8	+	++	+++	+++
Chenopodium quinoa	4	–	++	+++	+++
Circium arvense	2	–	++	++	++
	9	–	+	+	++
Convolvulus arvensis	2	–	+++	+++	+++
	4,6,8	–	++	+++	+++
Ipomoea hederacea	2	–	+++	+++	+++
	5,9	–	+	+++	+++
Ipomoea purpurea	2,5	–	++	+++	+++
Euphorbia esula	2,4	–	+++	+++	+++

[a] Conidial concentration in the IE was 1×10^8 conidia per ml. Greenhouse temperature range and relative humidity range were 22 to 30°C and 33 to 50%, respectively.

[b] – indicates no infection (disease index [DI] = 0); + indicates slight infection (DI = 0.1 to 2.9); ++ indicates some plants were severely infected and some were dead (DI = 3.0 to 3.9); and +++ indicates all plants were dead (DI = 4.0). See text for DI determination.

plied to the plants using a garden sprayer at temperatures equal to or greater than 25°C (77°F), but the IE carrier could not be applied easily by using the garden sprayer when the greenhouse temperature was 20°C because of increasing viscosity.

Effect of plant age and inoculum dose. The doses of *M. verrucaria* needed to kill weeds were correlated with plant age and the annual or perennial nature of each weed. Each 2-week-old plant was killed with 100 ml per m^2 of IE plus conidia, but 8- to 9-week-old plants required at least 200 ml per m^2 (Table 3). Annual and biennial weeds, such as redroot pigweed, Italian thistle, plumeless thistle, and common lamb's-quarters were killed by one application. Perennial weeds 6 weeks old or older, such as Canada thistle, field bindweed, and leafy spurge, produced new shoots from the stem portions or underground roots of the plants that were not killed by the pathogen. Repeated inoculations of the newly formed shoots were necessary to kill these perennial plants.

DISCUSSION

This report describes 42 new plant species in 31 genera and 8 families infected by *M. verrucaria* using artificial inoculation in the greenhouse. Twenty-seven of the 42 plant species were severely infected or killed, while the rest were slightly infected. The plants were inoculated with a very high conidial concentration (10^8 conidia per ml) until run-off and maintained in a dew chamber at 30°C for 18 h. Such conditions are rarely found in nature, which may partially explain why *M. verrucaria* is not a widespread plant pathogen although it is universally isolated (6).

This study confirms our previous finding (18) that dew temperature greatly enhanced the susceptibility of leafy spurge to *M. verrucaria*. In addition, redroot pigweed, curly dock, spinach, and potato were severely infected by *M. verrucaria* at 30°C dew temperature, but not at 25°C or lower dew temperatures. This pathogen severely infected or killed sunflower, field bindweed, alfalfa, and wild buckwheat at 30°C dew temperature, but caused only slight infection at 25°C or lower dew temperatures.

Our host range study confirms earlier reports that *M. verrucaria* was pathogenic on alfalfa, corn, cucumber, leafy spurge, peanut, red clover, rice, soybean, upland cotton, reed canarygrass, sunflower, and tomato. All 12 plant species, except peanut, rice, and tomato, were susceptible when the inoculated plants were incubated in the dew chamber at 30°C for 18 h but none were infected when the inoculated plants were kept in the dew chamber at or below 25°C.

The effects of prewounding host tissues on pathogenicity of *M. verrucaria* are conflicting (3–5). Our results support Braverman (3), but not Cunfer et al. (4) or de Romano (5), that prewounding is not necessary for infection of many plant species. Pathogenicity of *M. verrucaria* was enhanced by high dew temperature rather than prewounding. Wensley (13) also found that *M. verrucaria* was pathogenic to the roots of peach seedlings only at high temperatures. In addition to the effect of higher dew temperatures, IE formulation also increased the host species infected and severity of disease on plants (Table 2).

Myrothecium verrucaria from leafy spurge was effective in controlling annual and biennial plants, such as ivyleaf morning-glory, common lamb's-quarters, Italian thistle, and plumeless thistle, but not the perennials, such as Canada thistle, field bindweed, and leafy spurge, since *M. verrucaria* infection was not systemic, and the severely injured or killed perennial plants often recovered from the infection by production of new shoots from underground root buds or stem buds. Although *M. verrucaria* has mycoherbicide properties to control annual and biennial weed plants, its usefulness is questionable due to its wide host range. However, the pathogen may be studied as a broad spectrum mycoherbicide and tested in small plots since it is (i) worldwide in distribution and found in the air, soil, and plant material (6), (ii) a weak competitor in the soil and easily killed by other microorganisms (6), (iii) a pathogen of more than 12 crop plants but with no economic importance, (iv) not spread from diseased plants to healthy plants (18), and (v) required to be sprayed directly onto target weed to kill the weed. Pathogenicity was influenced by conidial concentrations and dew temperatures, but under natural conditions, high conidial concentrations and a dew period of 18 h at 30°C rarely exist. Precautions should be taken not to spray the pathogen directly onto economically important plants if tests are to be conducted in field plots.

The improved IE can be applied to plants using a garden sprayer at temperature equal to or greater than 25°C (77°F). But a large quantity of IE (10^3 liters per ha = 106 gallons per acre) was required to severely infect or kill 6-week-old or younger redroot pigweed, thistles, and common lamb's-quarters in the absence of dew. Further improvement of the formulation is needed in order to deliver the fungal pathogen to target weeds at 20°C (68°F) or lower temperatures and use less carrier.

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