Factors Influencing Pathogenicity of *Myrothecium verrucaria* Isolated from *Euphorbia esula* on Species of *Euphorbia*

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ABSTRACT

Yang, S.-M., and Jong, S. C. 1995. Factors influencing pathogenicity of *Myrothecium verrucaria* isolated from *Euphorbia esula* on species of *Euphorbia*. Plant Dis. 79:998-1002.

Leafy spurge (*Euphorbia esula*) inoculated with *Myrothecium verrucaria* had dead tops or shoots with blackened stems and blackened, curled, or wilted leaves. On leaves, circular spots with light brown centers and dark brown margins were produced. Pathogenicity was greatly affected by the conidial concentration and dew temperature, but not by the age of cultures from which conidia were used for inoculation. Conidia stored in sucrose solution at 4°C were viable for more than 14 months. *Myrothecium verrucaria* did not spread from diseased plants to healthy plants, even when the healthy plants touched the diseased plants in the dew tent for 7 days. Four-week-old or younger seedlings of leafy spurge grown from seeds were killed with one inoculation, but repeated inoculations were required to kill older plants. Pathogenicity tests on 89 collections of nine species of *Euphorbia* and on reed canarygrass (*Phalaris arundinacea*) showed that the pathogen severely infected all collections tested. The susceptibility of *E. corollata, E. cyathophora, E. cyparissias, E. helioscopia, E. heterophylla, E. lathyrus, E. marginata* Pursh *varigata, E. virgata,* and reed canarygrass to *M. verrucaria* is reported for the first time.

Additional keywords: host, invert emulsion

Leafy spurge (*Euphorbia esula* L.) is a herbaceous, deep-rooted perennial weed of the rangelands in North America and is a polymorphic complex of many biotypes (7). This weed infests over 1 million ha in the Northern Great Plains (6) and is poisonous to some animals. The economic losses due to the weed are estimated to be more than $50 million annually in the United States (11). Control of the weed with chemicals is both difficult and expensive, since the most effective herbicides often cannot be used near other desirable broadleaf species. Biological control using plant pathogens is being considered as a potential control method.

Myrothecium verrucaria (Albertini & Schwein.) Ditmar:Fr., isolated from leafy spurge collected in China in 1990, can kill leafy spurge in the greenhouse (10,14). Cunfer et al. (2) reported that an isolate of *M. verrucaria* from reed canarygrass (*Phalaris arundinacea* L.) was nonpathogenic to the host plant. Because of the lack of information on symptoms, factors influencing pathogenicity on leafy spurge, viability of conidia, spread of the pathogen, and pathogenicity on species of *Euphorbia* and reed canarygrass, this study was initiated to (i) describe the symptoms of disease, (ii) study the effect of conidial concentration, dew temperature, and culture age on disease development, (iii) determine the viability of conidia, number of inoculations required to kill the leafy spurge, and disease spread, and (iv) determine the pathogenicity on different accessions of *Euphorbia* species and reed canarygrass growing in our laboratory.

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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Accepted for publication 8 June 1995.

MATERIALS AND METHODS

Plant materials. All species except *Euphorbia cyparissias* L., *E. esula* L., *E. marginata* Pursh *varigata,* and *E. virgata* Waldst. & Kit. were grown from seeds. The four *Euphorbia* species were propagated from roots. The quantity and length of roots and the number of root buds varied from pot to pot and also among replications. The plants were grown in a pasteurized greenhouse soil mix as previously described (11). Each pot contained one seed-propagated plant, 5 to 10 tillers with reed canarygrass, and 1 to 5 shoots for root-propagated plants. Leafy spurge used in this study was FR-1 (Rock Co., Nebr.) or FR-9 (Sheldon Tall, N.D.), unless otherwise stated. All plants were used 3 to 4 weeks after planting, unless otherwise stated, and watered as required.

Inoculum production. A single-spore culture of *M. verrucaria* (10,14) from leafy spurge collected at E Qi Horn Hai Tzu Shiang, Jang Gang Twu Tsuen in Dongsheng, Inner Mongolia, People's Republic of China, in 1990 and designated 90C-56-5-2 (ATCC 90310) was used in this study. Four additional isolates of *M. verrucaria*, ATCC 22798, ATCC 26265, ATCC 36872, and ATCC 52802, were also used in some of the studies. Inoculum was produced and described as previously reported (13).

Symptoms. Symptoms on severely infected leafy spurge plants were described from the field specimens and greenhouse plants inoculated with ATCC 90310. Symptoms on slightly infected leaves were described from greenhouse inoculated plants because no field specimens were collected.

Effect of culture age and disease development. Potato-dextrose agar (PDA) plates were seeded with conidia of ATCC 90310 every week for 14 weeks and the plates incubated at 22±2°C with a 12-h photoperiod (40 $\mu E\ s^{-1}\ m^{-2}$ intensity). At 15 weeks, conidial suspensions prepared from 1- to 15-week-old cultures were atomized to leafy spurge (1 to 4 plants per pot) from 20 cm away (70.031×10^2 kg/m^2 [= 10 lbs./in^2]) until run off. All inoculated plants were first incubated in non-lighted dew chambers at 30°C for 18 h and then moved to greenhouse benches (20 to 32°C, and 33 to 64% relative humidity [RH]). After 13 days, disease severity was rated. Plants inoculated with the sterile aqueous sucrose solution served as controls. Each treatment had five pots of spurge (1 to 5 plants per pot) and each test was repeated once.

A 0 to 4 numerical system was used to rate the disease severity where 0 = no infection, no lesions on leaves; 1 = tip of leaves curled, brown, or less than 10 spots, not coalesced, on a leaf; 2 = more than 10 spots on a leaf, spots on leaves coalesced, some leaves yellow or brown, or lower leaves dropped but plant top still green; 3 = plant top brown or killed with leaves turned brown and defoliation but lower portions of stems still green, or for reed canarygrass, more than 50% of the leaves dead; 4 = whole plants dying or dead. A disease index (DI) score was then calculated by (summation of [severity rating ×

number of plants in that class)/total number of plants. An index below 3.0 indicated slight-to-moderate infection (resistant), but at or above 3.0 indicated severe infection (susceptible). Severity of injuries by the invert emulsion alone was also taken at the same time disease severity was taken. Following severity rating, attempts were made to reisolate the pathogen on PDA from the infected tissues. The tissue pieces cut from the infected plants were surface disinfected in 2.5% NaOCl solution for 5 to 10 min, washed in sterile distilled water for 5 to 10 min, placed on PDA plates, and incubated at 30°C for at least 1 week. Presence of sporodochia and conidia of *Myrothecium* on the PDA indicated a positive infection of the inoculated plants.

Conidia viability. Viability of conidia of ATCC 90310 stored in 2% sucrose solution at 4°C was inoculated onto five pots of leafy spurge once a month for 14 months. Infection of the plants indicated that the spores were viable. Viability of conidia stored at 4°C after 1 and 14 months were also determined by testing the germination of conidia on PDA plates. This test was repeated once.

Effect of conidial concentration on disease development. Conidial concentrations of ATCC 90310, ATCC 22798, and ATCC 36872 ranging from 10^1 to 10^8 conidia per ml were separately atomized to five pots of leafy spurge plants and the test was repeated once.

Dew temperature and disease development. Leafy spurge plants inoculated with ATCC 90310, ATCC 22798, and ATCC 36872 were incubated in dew chambers in darkness at 25 and 30°C for 18 h and at 10, 15, and 20°C for 18 or 72 h and then moved to the greenhouse and rated, as described.

Number of inoculations to kill leafy spurge. Leafy spurge was grown in 20-cm clay pots from seeds or root pieces as described. For the first inoculation, the number of shoots per pot varied from 1 to 16. However, in the second and subsequent inoculations the number of shoots per pot varied from 1 to 37, including the old and new shoots. Times from planting roots and seed until first inoculation were <4, 4, 6, 10, and 15 weeks. For each age, there were five pots and the test was repeated once. Plants were inoculated with conidial suspensions of ATCC 90310 and incubated, as described. New and old shoots in pots were inoculated every 2 to 3 weeks until no new shoots emerged either from the soil or stems. Fifteen-week-old leafy spurge plants were also separately inoculated with the other four ATCC isolates of *M. verrucaria.*

Spread of pathogen in the greenhouse. Spread of pathogen was studied in a dew chamber at 30°C and dew tent at 25 to 30°C. Two weeks after inoculation with conidia of isolate ATCC 90310 or ATCC 36872, leafy spurge plants were severely infected or killed, but new shoots (5 to 10 cm in height) had emerged from the dead plants. These pots were then incubated in the dew chamber again for 48 h once a week for 2 weeks. The healthy plants were adjacent to diseased plants since pots were arranged closely in the dew chamber. In a second experiment, leafy spurge plants and healthy seedlings (4-week-old) were incubated in the dew tent for a week with a continuously running humidifier (Walton Humidifier, Walton Laboratories Inc., Irvington, N.J.) (98 to 100% RH). The healthy plants were also adjacent to diseased plants. They were moved to greenhouse benches for another 2 weeks before being examined for infection.

Infection of leafy spurge in absence of dew. Invert emulsion carrier (IEC) was prepared, as previously described (12). Conidia were suspended in the aqueous sucrose solution before mixing with the oil phase. Final conidial concentration in the IEC or aqueous sucrose solution used for inoculation was 1×10^8 conidia per ml. One hundred milliliters of IEC plus conidia of ATCC 90310, ATCC 22798, and ATCC 36872 was separately sprayed using a 1-gal garden sprayer (12). Control plants were sprayed with IEC alone and aqueous sucrose solution plus conidia. In addition, plants inoculated with aqueous sucrose solution plus conidia placed in dew chamber at 30°C for 18 h after inoculation were also included in each test. Each treatment had 10 pots of 4-week-old plants (5 to 10 shoots per pot) and this test was repeated four times with isolate ATCC 90310 and once with the other two isolates. Inoculated plants were kept on greenhouse benches (20 to 32°C, 40 to 64% RH) and rated 2 weeks after inoculation. Plants were watered when required but precautions were taken not to wet the aboveground parts of plants after inoculation. Duncan's new multiple range test was used to compare the means of the four treatments.

Pathogenicity of *M. verrucaria* on different species of *Euphorbia* and reed canarygrass. A reed canarygrass from Nebraska and 89 accessions in nine species of *Euphorbia* from China, Europe, and North America were atomized with aqueous sucrose solution (control) or aqueous conidial suspension of ATCC 90310 as described above.

RESULTS

Symptoms. Symptoms on the diseased plants collected in Dongshen, Inner Mongolia, China, consisted of dead shoots with blackened stems and blackened, curled, or wilted leaves. Sporodochia with white mycelia developed when stems and leaves were placed on moistened filter paper in petri dishes and incubated at 30°C for 18 h. Greenhouse symptoms on slightly infected leaves were circular spots (0.2 to 0.7 cm in diameter) with light brown centers and dark brown margins. Severely infected plants had dead shoots or dead tops with blackened stems and brown-to-black, curled, and wilted leaves.

Effect of culture age on disease development. Conidia were formed the following day after PDA plates were seeded with conidia but not mycelium of ATCC 90310 at 30°C. Conidia were produced continuously and covered most of the colonies within 7 days. Conidia from 1- to 15-week-old cultures showed no differences in infection of leafy spurge since all inoculated plants were severely infected or killed (DI > 3.6) regardless of the source of conidia if the plants were incubated in the dew chamber at 30°C for 18 h. The pathogen was easily reisolated from the severely infected plants.

Conidia viability. Conidia of ATCC 90310 stored in aqueous sucrose solution for 14 months still infected or killed leafy spurge. No spores were germinated in sucrose solution during storage. Ninety-three and 82% of spores (average of two tests) stored for 1 and 14 months, respectively, germinated after two drops of spore suspensions were placed on PDA plates for 8 h.

Effect of conidial concentration on disease development. All the leafy spurge plants sprayed with conidial suspensions of ATCC 90310, ATCC 22798, or ATCC 36872 at 8×10^7 conidia per ml and greater (1×10^8 conidia per ml) were severely infected or killed. However, disease severity on leafy spurge sprayed with 1 to 4×10^7 conidia per ml was inconsistent: some plants were severely infected (rated as 3), but other plants were not infected (rated as 0). Plants sprayed with 10^6 conidia per ml showed slight infection (rated as 2) to no infection but no infection at 10^5 per ml or lower. No disease occurred on plants inoculated with aqueous sucrose solution. The pathogen was consistently reisolated on PDA from the severely infected leaves and stems.

Dew temperature and disease development. Temperatures during the dew period significantly affected disease severity. The leafy spurge plants inoculated with ATCC 90310 and incubated in the dew chamber at 30°C for 18 h were severely infected or killed (DI > 3.5, 100% infection and disease severity rated 3 to 4), but plants incubated in the dew chamber at 25°C were slightly to severely infected (DI < 2.5, 100% infection and disease severity rated from 1 to 3). No infection occurred when the inoculated plants were incubated in the dew chamber at or below 20°C for 18 h. However, slight infection occurred on some plants at 20°C but not at 15°C for 72 h (DI < 1.5 at 20°C, 65% of plant infected and disease severity rated from 0 to 2). The other two ATCC isolates (22798 and 36872) also severely infected and killed those plants that had been incubated

in the dew chamber at 30°C for 18 h, slightly to severely infected those inoculated plants at 25°C, and caused no infection on those inoculated plants incubated at or below 20°C for 18 h.

Spread of pathogen in the greenhouse. The pathogen did not spread from diseased leafy spurge plants to healthy plants since no disease developed 2 weeks after healthy plants touched severely infected plants during incubation in the dew chamber for 48 h twice in 2 weeks, or for 7 days in the dew tent.

Number of inoculations to kill leafy spurge. Isolate ATCC 90310 of *M. verrucaria* could kill all the leafy spurge regardless of plant age if new sprouts and surviving older plants were reinoculated every 2 to 3 weeks. The number of inoculations needed to kill all plants in 10 pots in two experiments depended on plant age and source, i.e., grown from seed or root pieces at the time of the first inoculation. Vigorously growing old plants with many shoots and root buds required more inoculations than weakly growing plants with few shoots and root buds. Plants grown from seeds for 4 weeks or less were killed (no pots producing new shoots 3 weeks after inoculation) with one inoculation but plants grown from root pieces required 10 inoculations. Six-week-old plants grown from seeds and root buds were killed with 13 and 14 inoculations, respectively. Ten- and 15-week-old plants grown from seeds were killed with 16 and 18 inoculations, respectively, while 10- and 15-week-old plants grown from root pieces were killed with 17 and 20 inoculations, respectively.

New shoots rapidly grew from undamaged portions of stem and also from the underground root buds when tops (top of stems or aboveground portions of plants) were severely injured or killed by the pathogen after each inoculation. The number of new shoots per pot that grew from stems or underground roots ranged from 1 to 37 after each inoculation and the number of new shoots increased or decreased varied from pot to pot. Repeated inoculations of the new and old shoots at 2- to 3-week intervals eventually killed the plants in pots.

The other four isolates of *M. verrucaria*, ATCC 22798, ATCC 36872, ATCC 2625, and ATCC 52802, also required 18 to 21 inoculations to kill the 15-week-old leafy spurge growing in pots.

Infection of leafy spurge in absence of dew. Isolate ATCC 90310 severely infected or killed all plants in the absence of dew when the conidia were suspended in IEC and sprayed onto the plants. Some control plants sprayed with IEC alone were slightly injured: some leaves turned brown and dropped. Plants sprayed with aqueous conidial suspensions and incubated in the greenhouse without dew showed no infection. Plants sprayed with the aqueous conidial suspensions and incubated in a dew chamber were severely infected or killed as were those inoculated with the conidia suspended in IEC without dew (Table 1). The difference was not statistically significant ($P = 0.05$). Results of inoculations with ATCC 90310 are shown in Table 1. The other two isolates (ATCC 22798 and ATCC 39872), like the isolate ATCC 90310, also effectively killed leafy spurge in the absence of dew. The pathogen was reisolated from the infected plants but not from the damaged control plants.

Pathogenicity of *M. verrucaria* on different species of *Euphorbia* and reed canarygrass. All 89 accessions in the nine species of *Euphorbia* were susceptible to *M. verrucaria* isolate ATCC 90310 (Table 2). *Euphorbia cyparissias* L. and *E. esula* from the United States were as susceptible to *M. verrucaria* as those from the other countries. *Euphorbia virgata* Waldst. & Kit from Azerbaizan, Dagestan, and Russia were as susceptible as those from Hungary, Italy, the former Czechoslovakia, and Romania. *Euphorbia virgata* has not been found in the United States.

Although aboveground portions of *E. cyparissias, E. esula*, and *E. virgata* were killed by *M. verrucaria*, new shoots developed from the underground buds or from the portion of stems not killed. The control plants remained uninfected. This pathogen was reisolated from the infected plants.

Myrothecium verrucaria severely infected (disease severity of 3) but did not kill the reed canarygrass. The leaf blades (50% of leaves) turned brown to black and died. Control leafy spurge and reed canarygrass plants were not infected. *Myrothecium verrucaria* was reisolated from the infected reed canarygrass and leafy spurge.

Table 1. Effect of invert emulsion on infection of leafy spurge by *Myrothecium verrucaria* (ATCC 90310)

Treatment	Disease index[a]					
	Replications					
	I	II	III	IV	V	Mean
Invert emulsion[b]	1.6	0.1	0.5	0.5	1.4	0.8 b[c]
Invert emulsion + *M. verrucaria*	3.4	3.5	3.1	3.7	4.0	3.5 a
Aqueous sucrose solution + *M. verrucaria*[d]	0	0	0	0	0	0 c
Aqueous sucrose solution + *M. verrucaria*[e]	4.0	4.0	3.8	3.9	3.7	3.9 a

[a] Disease index indicates the severity of disease, which was rated on a scale from 0 (no infection) to 4 (dead plants).

[b] Plants inoculated with invert emulsion only or invert emulsion plus *M. verrucaria* were placed in the greenhouse at 20 to 32°C, and 40 to 64% relative humidity (no dew period).

[c] Numbers followed by the same letter in the last column are not significantly different according to Duncan's new multiple range test ($P = 0.05$). Average of 50 pots in each treatment, 10 pots for each test.

[d] Inoculated plants were also placed in the greenhouse after inoculation (no dew period).

[e] Inoculated plants were first incubated in a dew chamber at 30°C for 18 h and then moved to the greenhouse.

DISCUSSION

This is the first description of symptoms caused by *M. verrucaria* on leafy spurge. Symptoms on slightly infected leaves were similar to those on red clover (*Trifolium pratense* L.) (3) and birdsfoot trefoil (*Lotus corniculatus* L.) (2): light brown center with dark brown margins. However, severely infected leafy spurge died or had dead top portions of stem with blackened stems and brown black and curled leaves.

Myrothecium verrucaria severely infected 89 collections of *Euphorbia* spp and reed canarygrass. This is the first report of *M. verrucaria* pathogenic on eight species of *Euphorbia* and reed canarygrass. Our isolate from leafy spurge was different from the isolate obtained from reed canarygrass by Cunfer et al. (2) who reported that their isolate infected red clover (*Trifolium pratense* L.) but not reed canarygrass. Our isolate also severely infected red clover (13).

Myrothecium verrucaria produces many conidia on infected plants but they might not germinate and infect new plants in the greenhouse because of conidia or because germination of the profusely produced conidia is self-inhibitory (3).

To severely infect or kill leafy spurge, a large conidial concentration was required and the inoculated plants had to be incubated in a dew chamber at 30°C for 18 h if an IEC was not used. Conidia from 1-week-old cultures grown on PDA were as pathogenic as those from older cultures. The conidia had a shelf life at 4°C of more than 14 months and the conidia did not germinate during storage.

Isolates ATCC 22798, ATCC 36872, and ATCC 26265 of *M. verrucaria* were respectively isolated from peanut (*Arachis hypogaea* L.), soybean (*Glycine max* (L.) Merr.) and wheat (*Triticum aestivum* L.) rhizosphere (1). The origin of the isolate ATCC 52802 is unknown (1). However, all four isolates were pathogenic and could kill leafy spurge. This is the first report of leafy spurge as a host of these. The pathogen may infect many plant species when the plants are inoculated with a large number of conidia and placed in the dew chamber at 30°C for 18 h. Yang and Jong (13) have found isolate ATCC 90310 of *M. verrucaria* pathogenic on many species of plants. Conidial concentrations and dew

temperatures are the limiting factors for development of disease on leafy spurge in the greenhouse. Dew period and temperature were overcome by using an IEC. Therefore, *M. verrucaria*, like *Alternaria alternata* (Fr:Fr.) Keissl. and *A. angustiovoidea* E. Simmons (12), can infect leafy spurge without dew in the greenhouse (20 to 32°C, 40 to 64% RH).

Myrothecium verrucaria could severely infect leafy spurge, but the severely infected plants often recovered by production of new shoots from underground root buds or stem buds. Repeated inoculation of new shoots was required to kill the plants. The number of inoculations required to kill the established leafy spurge was dependent on the number of viable buds underground and also on undamaged stem portions at time of inoculation. Because *M. verrucaria* infection was not systemic, plants that died after repeated inoculation probably died from lack of root buds or photosynthate rather than because of the direct action of the pathogen. Since *M. verrucaria* was ineffective in killing all established plants with root buds, the organism has little potential as a biological control pathogen for leafy spurge.

However, this pathogen has the following properties of a useful mycoherbicide: worldwide distribution (3,4); rapid production of conidia; a weak competitor in soil (5,8) and short period of survival in plant residues (S.-M. Yang, *unpublished data*); shelf life in sucrose solution at 4°C for more than a year; inability to spread from diseased plants to healthy plants; and ability to infect and kill plants in the absence of dew when conidia is applied with a carrier to the plants. Although the pathogen does not effectively control perennial leafy spurge, it may effectively kill annual weeds. Preliminary studies on controlling the annual or biennial plumeless thistle (*Carduus acanthoides* L.) with two isolates of *M. verrucaria* (ATCC 90310 from leafy spurge and ATCC 18398 from submerged balsa wood in Maryland) in the greenhouse have been reported (9). Further studies are needed concerning the development of the pathogen as a mycoherbicide to control annual weeds and biennial weeds.

Table 2. Infection of different *Euphorbia* species from different collection sites by *Myrothecium verrucaria* (ATCC 90310) in the greenhouse[a]

Euphorbia species and accession number	Collection sites[b]		Year collected	Disease index[c]
	City or county	State or country		
E. corollata L.				
FR -59	San Bernadino	Calif.	1990	4.0
FR-50	Menetor	Ohio	1990	4.0
FR-34	Mission	Tex.	1990	4.0
E. cyathophora Murr.				
FR-26	Cameron Co.	Tex.	1989	3.0
E. cyparissias L.				
FR-27	?	Md.	1982	4.0
FR-70	?	N.J.	1990	3.0
FR-78	Loudoun Co.	Va.	1991	3.0
FR-32	Jefferson Co.	W.Va.	1982	4.0
FR-120 +1[d]	Laaben	Austria	1991	3.4
FR-122 +1[d]	Gyor	Hungary	1991	3.0
FR-114	Nanesti	Romania	?	3.3
E. esula L. (from U.S.)				
FR-66	Meeker	Colo.	1990	4.0
FR-14	Butte Co.	Idaho	1978	3.3
FR-75	Delaware Co.	Ia.	1991	4.0
FR-133 +2[d]	Garrett Co.	Md.	1992	4.0
FR-15	Kalkaska Co.	Mich.	1978	4.0
FR-16	Becker Co.	Minn.	?	3.0
FR-7 +2[e]	Gallatin Co.	Mont.	1989	4.0
FR-1 +11[e]	Rock Co.	Nebr.	1989	4.0
FR-9 +4[e]	Sheldon Tall	N.D.	1978	4.0
FR-18	Baker Co.	Oreg.	1978	4.0
FR-28	Pike Co.	Pa.	1982	4.0
FR-139	Vermillion	S.D.	1990	4.0
FR-23	Hidalgo Co.	Tex.	1989	3.0
FR-135 +1[d]	Grant Co.	W.Va.	1992	4.0
FR-4 +1[d]	Sundance	Wyo.	1989	4.0
E. esula (not from U.S.)				
FR-12 +3[e]	Krems	Austria	1978	4.0
FR-42	Kamloops	B.C., Canada	1978	4.0
FR-49	Dongshen	China	1989	4.0
FR-44 +1[e]	Mazaszaszar	Hungary	1982	4.0
FR-39 +2[e]	Pisa	Italy	1986	3.0
FR-92	Stavropol	Russia	1991	4.0
FR-53 +1[e]	?	Turkey	?	4.0
FR-48	?	Yugoslavia	1982	4.0
E. helioscopia L.				
FR-140	?	?	1981	4.0
E. heterophylla L.				
FR-25	?	Netherlands	1989	3.0
E. lathyrus L.				
FR-57	Manassas	Va.	1990	3.0
E. marginata Pursh *varigata*				
FR-24	?	Netherlands	1989	4.0
E. virgata Waldst. & Kit.				
FR-103 +2[d]	Crymora	Azerbaizan	1991	4.0
FR-130 +1[d]	Iza	Czechoslovakia	1991	4.0
FR-124	Parad	Hungary	1991	4.0
FR-116 +4[d]	Galati	Romania	1991	4.0
FR-100 +4[d]	Mahuchkala	Dagestan Russia	1991	3.0
FR-104	Sezgokala	Dagestan Russia	1991	4.0
FR-38 +3[e]	Stavropol	Russia	1991	4.0

[a] Inoculated plants were incubated in a dew chamber at 30°C for 18 h and then maintained in a greenhouse (24 to 30°C, 30 to 50% relative humidity) for 14 days.
[b] ? indicates unknown city, county, country, or collection year.
[c] Disease severity was rated from 0 to 4, where 0 = no disease and 4 = plants dying or dead 2 weeks after inoculation. Disease index was calculated from summation of (severity rating × number of plants in the rating)/total number of plants. Average of 20 plants in two tests. Conidial suspension of *M. verrucaria* was 1×10^8 conidia per ml.
[d] Number indicates additional accessions from the same city or country collected in the same year.
[e] Number indicates additional accessions from different cities or countries in different years.

LITERATURE CITED

1. Anonymous. 1991. ATCC Filamentous Fungi. 18th ed. ATCC, Rockville, Md.
2. Cunfer, B. M., Graham, J. H., and Lukezic, F. L. 1969. Studies on the biology of *Myrothecium roridum* and *M. verrucaria* pathogenic on red clover. Phytopathology 59:1306-1309.
3. Domsch, K. H., Gams W., and Anderson, T. H. 1980. Compendium of Soil Fungi. Vol. 1. Academic Press, New York.
4. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul, Minn.
5. Grosu, R. and Hulea, A. 1973. Morphogenesis, sporogenesis, and cultural and physiological features of *Myrothecium verrucaria*. Rev. Roum. Biol. Ser. Bot. 18:219-225.
6. Hanson, T., Kirby, D., Sieg Hull, C., and Potts, L. 1992. Biological control of leafy spurge using Angora Goats. Pages 53-54 in: Proc. Leafy Spurge Symp. Sect. 2. Great Plains Agric. Council Publ. No. 144. University of Nebraska, Lincoln.
7. Lacey, C. A., Kott, R. W., and Fay, P. K. 1984. Rancher control leafy spurge. Rangelands 6: 202-204.
8. Martin, J. P., Ervin, J. O., and Shepherd, R. A.

1959. Decomposition and aggregating effect of fungus cell material in soil. Proc. Soil Sci. Soc. Am. 23:217-220.
9. Yang, S. M. 1994. Control of *Carduus acanthoides* with *Myrothecium verrucaria* in the greenhouse in the absence of dew. (Abstract). Phytopathology 84:1136.
10. Yang, S. M., Dowler, W. M., and Johnson, D. R. 1991. Comparison of methods for selecting fungi pathogenic to leafy spurge. Plant Dis. 75:1201-1203.
11. Yang, S. M., Johnson, D. R., and Dowler, W. M. 1990. Pathogenicity of *Alternaria angustiovoidea* on leafy spurge. Plant Dis. 74:601-604.
12. Yang, S.-M., Johnson, D. R., Dowler, W. M., and Connick, W. J., Jr. 1993. Infection of leafy spurge by *Alternaria alternata* and *A. angustiovoidea* in the absence of dew. Phytopathology 83:953-958.
13. Yang, S.-M., and Jong, S. C. 1995. Host range determination of *Myrothecium verrucaria* isolated from leafy spurge. Plant Dis. 79:994-997.
14. Yang, S.-M., Zhuang, J.-Y., and Liu, W.-J. 1993. Pathogens of leafy spurge in Inner Mongolia, China. Plant Dis. 77:319.