Relative Virulence of *Melampsora euphorbiae* from Central Europe Toward North American and European Spurges

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ABSTRACT

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Isolates of *Melampsora euphorbiae* collected in Austria, Hungary, Italy, and Switzerland were evaluated for virulence and specificity toward leafy and cypress spurges. Host specificity studies conducted for five of these isolates revealed that each isolate was most virulent on the collection of leafy or cypress spurge from which it came, and one isolate was restricted to its original collection of leafy spurge. Two of the isolates infected both leafy and cypress spurge, and though each was highly virulent toward the species of origin, it was only weakly virulent on the other. Three of the five isolates also infected a few North American collections of leafy or cypress spurge, but disease severity was very low in each instance. Reasons for desirability to collect *M. euphorbiae* from other geographical locations are given.

Additional key words: biological weed control, *Euphorbia* sp.

Leafy spurge is a perennial weed introduced from Eurasia that infests about one million hectares of rangeland and pastureland in North America (4). In North Dakota alone, it incurs an estimated $12.9 million loss per year (1982 data) in red meat production and implementation of control measures (10). According to Dunn (4), the origin of leafy spurge, particularly in the Great Plains region where it is most serious, is probably the Ukraine Province and the Volga River valley in Russia.

Leafy spurge in North America usually has been identified as *Euphorbia esula* L. or *E. virgata* Waldst. & Kit. (3,4). Recently, Ebke and McCarty (5) recognized five morphologically distinct leafy spurge taxa in collections from North America: *E.* × *pseudovirgata* (Schur) Soo, *E. esula, E. uralensis* Fisch. ex Link, *E. agraria* M. Bieb, and *E. cyparissias* L. A summary of other lists is given by Radcliffe-Smith (13), who reports that several of these species have been known to hybridize in Eurasia, including *E. cyparissias, E. esula,* and *E. agraria*. Hybridization between several of these species may be one explanation for the complexity of spurge taxonomy in North America (13).

Cypress spurge (*E. cyparissias*), a close relative of leafy spurge, was introduced as an ornamental from Europe. It has become naturalized and is recognized as a weed in parts of eastern North America (3,6).

Control of leafy and cypress spurges by conventional methods has not been successful. Plants of each species have extensive root systems that enable them to survive and spread despite repeated chemical treatments and tillage operations. They also occur where limited monetary returns restrict the amount of money that can be justified for control (17).

Attempts to use insect natural enemies for classical biological control of leafy spurge have not been successful either. Since 1965, two of three European insect species have become established on leafy spurge in North America, but no significant reduction in stands has been reported to date (6). Harris (6) speculates that biological differences may exist between leafy spurge from Russia and western or central Europe, and for this reason, insects from the latter region may not adapt to the spurge that occurs in the Great Plains.

Several plant pathogens have been reported on leafy and cypress spurge in

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North America (1,2,15), but none of these prevents spurge from spreading, and none appears promising in augmentative biological control. All of the rust fungi attacking leafy spurge in North America have alternate hosts in the genera *Medicago* and *Lupinus* of the Leguminosae (1,2). *Melampsora euphorbiae* (Schub.) Cast., an autoecious rust fungus, has been reported in North America on cypress spurge but not on leafy spurge (15). Of the other fungi known to attack leafy spurge, only a leaf-spotting *Alternaria* sp. has shown any potential (8,11). Considerable manipulation of *Alternaria* will be required for it to be effective, because it is not present naturally at levels that reduce stands of spurge (11).

Because of the need for low-cost, effective control of leafy and cypress spurges in North America, a search for host-specific, virulent plant pathogens was initiated. *M. euphorbiae* was considered a good candidate, because it is autoecious and strains have been reported that are restricted to a few species of *Euphorbia* (12). Since 1980, pathogens of leafy and cypress spurge have been collected as part of a cooperative research effort between the USDA Foreign Disease-Weed Science Research Unit (FDWSRU) and the Institut für Phytomedizin, Eidgenössische Technische Hochschule (ETH) in Zürich, Switzerland. An additional collection trip, supported by Montana State University, was made in August 1982. Forty-seven isolates of *M. euphorbiae* were obtained as a result of these efforts, 11 of which were established initially under greenhouse conditions. Five were evaluated for virulence and specificity toward leafy and cypress spurge. Results of evaluations are presented and the potential for *M. euphorbiae* in biological control of spurges in North America is discussed.

Table 1. Relative virulence of four isolates of *Melampsora euphorbiae* on leafy spurge and cypress spurge evaluated at the Foreign Disease-Weed Science Research Unit, Frederick, MD

	Plant collection		Isolate			
Species	**Desig.**	**Origin**	**20**	**27**	**30**	**32**
Leafy spurge[a]	AU 1	Inprugg, Austria	−[b]	−	+++[c]	−
	AU 2	Krems, Austria	···	···	···	−
	AU 3	Grossweikersdorf, Austria	···	−	···	···
	AU 10	Krems, Austria	···	−	−	−
	BC 25	Kamloops, BC, Canada	−	R	···	−
	H 9	Budapest, Hungary	···	R	···	−
	H 10	Mazaszaszar, Hungary	···	+++[c]	···	−
	I 1	Compito, Italy	+	+	···	−
	IA 9	Manchester, IA	+	−	···	−
	IA 19	Manchester, IA	+	···	···	−
	ID 5	Arco, ID	−	−	−	−
	MD 1	Bittinger, MD	−	R	···	−
	MI 36	Kalaska, MI	−	+	···	−
	MN 3	Becker County, MN	−	+	···	−
	MN 15	Shakopee, MN	−	−	−	−
	MN 17	Becker County, MN	−	−	−	−
	MN 18	Becker County, MN	−	−	···	−
	MN 19	Becker County, MN	−	−	−	−
	MT 1	Montana	···	−	−	−
	MT 2	Montana	−	+	−	−
	MT 3	Montana	−	+	−	−
	MT 4	Montana	−	−	−	−
	MT 5	Montana	−	−	−	−
	MT(s)	Montana (seed)	+	−	−	−
	ND 1	Sandhills, ND	···	···	···	−
	ND 5	North Dakota	···	···	···	−
	NE 1	Nebraska	−	R	···	−
	NE 12	Lincoln, NE	−	−	−	−
	NE 39	Chadron, NE	···	···	···	−
	NJ 1	Rutgers, NJ	−	+	−	−
	NJ 2	Middlebush, NJ	···	···	−	−
	NJ 3	Edison, NJ	···	−	···	−
	NV 8	Elko County, NV	+	+	−	−
	OR 1	Oregon	−	R	−	−
	OR 14	Baker, OR	−	−	···	−
	WY 1	Wyoming	−	−	−	−
	WY 9	Crook County, WY	−	−	···	−
Euphorbia cyparissias	CAU 23a	Griechenberg, Austria	++	···	···	−
	CAU 24	Nesselstauden, Austria	+++[c]	···	···	−
	CCH	Sufers, Switzerland	++	+	−	+++[c]
	CI 1	Compito, Italy	···	···	···	−
	CMD	Frederick, MD	+	−	−	−
	CMT	Montana	+	−	−	−
	CPA	Dingmans Ferry, PA	···	−	···	−

[a] Not identified to species.
[b] Plant reaction designations are as follows: ··· = not tested; − = immune, no macroscopic symptoms; R = red flecks or lesions, no sorus development, resistant; + = fewer than 10% of inoculated leaves infected, one or two sori per leaf; ++ = 10–35% of inoculated leaves infected, two to four sori per leaf; and +++ = 35–65% of inoculated leaves infected, four to 10 sori per leaf.
[c] Indicates host plant for particular isolate.

MATERIALS AND METHODS

Collections of *M. euphorbiae* were made in Austria, Hungary, Italy, and Switzerland between 1980 and 1982 by scientists from both ETH and FDWSRU. Whole infected plants were dug by scientists from ETH and transported to Zürich for study. Because of physical limitations in transporting entire infected plants to FDWSRU, infected leaves and stems were removed and air-dried before shipment. In most instances, crowns from infected plants also were dug, trimmed back, and transported to FDWSRU so compatible host tissue would be available for pathogen propagation and study. Pathogen and host materials were shipped to FDWSRU with appropriate permits, and all research at FDWSRU was carried out according to recommended guidelines (7) in a containment greenhouse (9).

Isolates established at each laboratory were increased for study under greenhouse conditions. At ETH, 4- to 7-wk-old rooted cuttings of leafy and cypress spurges were sprayed until thoroughly wet with 100,000 freshly harvested urediniospores per milliliter in water containing 0.01% Etalfix (25% Citowett [isooctylphenyl ether of polyethylene glycol] diluted in 20% methyl alcohol; Maag AG, Dielsdorf, Switzerland). Plants were then covered with a black plastic bag for 24 hr. A minimum of five plants in each of two replicates was inoculated from each location. Plants were maintained in a climatized greenhouse at day/night temperatures of 20/15 ± 2 C and were given supplemental fluorescent light in the winter to maintain a 16-hr photoperiod.

At FDWSRU, inoculum was transferred by hand directly from pustules to leaves of test plants and spread over the leaf surface by gentle rubbing with the index finger. A susceptible control plant was included for each inoculation. Inoculated plants were put in a dew chamber without light for 16–24 hr at 21 C. Inoculated plants were grown in a greenhouse at day/night temperatures of 23/18 ± 2 C and were given supplemental fluorescent light in the winter to maintain a 16-hr photoperiod. The number of plant collections inoculated in the evaluation of each isolate varied depending on the success rate for rooting collections of leafy and cypress spurge. When inoculations were carried out (Table 1), at least three plants from each

collection were used in each evaluation.

Examination for pustule development was made daily at both locations beginning 10 days after inoculation and continuing for the next 2 wk. The relative virulence of each isolate in this host specificity study was based on disease severity. A scale of − (immune, no macroscopic symptoms) to +++++ (more than 90% of inoculated leaves infected, more than 10 sori per leaf) was used to rate disease severity for each plant. A more precise evaluation of severity was not made because of the specificity observed for each isolate, and particularly because North American spurges generally were not susceptible to the isolates in this study.

Plant species inoculated in host specificity tests were nearly the same for the two laboratories. Tests at ETH involved 11 collections of leafy spurge from Europe and 10 from North America, three European collections of cypress spurge, and one collection each of *E. lathyris* L. and *Ricinus communis* L. The host specificity test at FDWSRU included seven leafy spurge collections from Europe and 30 from North America, four cypress spurge collections from Europe and three from North America, plants of eight other *Euphorbia* spp., and 24 crop or latex species. The expanded host specificity study involving the additional *Euphorbia*, crop, and latex species at FDWSRU used isolate E-32 and involved inoculation of at least four plants each in four 10-cm pots in the manner described.

RESULTS

Each isolate of *M. euphorbiae* was most virulent on the collection of leafy or cypress spurge from which it was obtained (Tables 1 and 2). At ETH, leafy spurge from Passo Abetone, Italy, was much more susceptible to isolate E-49 than the other spurge collections. Reaction of the host spurge collection from Passo Abetone to isolate E-49 was +++++, whereas plants from Mantova, Italy, about 100 km from Passo Abetone, were rated +++ (35–60% of the leaves infected, four to 10 sori per infected leaf). Susceptibility was less than +++ for the remainder of the leafy spurge collections evaluated at ETH, and no infection occurred on *E. cyparissias* or two other species inoculated with this isolate.

Four isolates evaluated at FDWSRU were moderately virulent on the European host plants from which they were collected (Table 1). Disease severity was always less on other European or North American collections of the same species than on the original host. Usually, reactions on the latter collections ranged from − to + (10% of the leaves infected, one or two sori per leaf). In two instances, however, plants of species other than the host also were infected. Isolate E-20, collected on *E. cyparissias*, was weakly virulent on five of 26 leafy spurge collections and weakly to moderately virulent (++) on two of four remaining collections of *E. cyparissias*. Isolate E-27, from leafy spurge, also was weakly virulent on one of four collections of cypress spurge and on seven of 30 collections of leafy spurge that were inoculated.

In all instances, infection did not occur on any species other than leafy or cypress spurge. The study at ETH indicated that isolate E-49 would not infect either *E. lathyris* or *R. communis* (Table 2). An expanded host specificity study using isolate E-32 at FDWSRU revealed that none of 21 crop, two latex, or eight additional *Euphorbia* spp. was susceptible to the pathogen.

The following crop and latex species were included in the evaluation at FDWSRU (asterisk indicates a species with latex): Asclepiadaceae: **Asclepias syriaca* L. (milkweed); Buxaceae: **Simmondsia chinensis* (Link) C. Schneid. (jojoba); Chenopodiaceae: *Beta vulgaris* L. (sugar beet), *Spinacia oleracea* L. (spinach); Compositae: *Carthamus tinctorius* L. (safflower), *Cynara scolymus* L. (artichoke), **Lactuca sativa* L. (lettuce); Cruciferae: *Brassica oleracea* L. (cabbage); Cucurbitaceae: *Cucumis sativus* L. (cucumber); Gramineae: *Avena sativa* L. (oat), *Hordeum vulgare* L. (barley), *Oryza sativa* L. (rice), *Panicum miliaceum* L. (millet), *Secale cereale* L. (rye), *Sorghum bicolor* (L.) Moench (sorghum), *Triticum aestivum* L. (wheat), *Zea mays* L. (corn); Leguminosae: *Glycine max* (L.) Merr. (soybean), *Medicago sativa* L. (alfalfa), *Phaseolus lunatus* L. (lima bean), *Trifolium pratense* L. (red clover); Liliaceae: *Allium cepa* L. (onion).

Euphorbia spp. related to leafy and cypress spurges and included in the evaluations were *E. corolata* L., *E. heterophylla* L., *E. lathyris, E. maculata* L., *E. peplus* L., *E. prostrata* Ait., *E. pulcherrima* Willd. ex Klotzsch, and *E. tirucalli* L.

DISCUSSION

Plant pathogens evaluated for biological control of noxious weeds ideally must be found both safe to use around nontarget plants and virulent on the target species (16). Results from this study indicate that *M. euphorbiae* can be virulent toward specific collections of the host and sufficiently restricted in host range to be considered for biological control of leafy spurge. Results from the present study indicate isolates of *M. euphorbiae* from leafy and cypress spurge are restricted to the subgenus *Esula* section *Esula*, following the scheme in *Flora Europaea* (14). *Euphorbia* spp. in four other subgenera and two other sections of the subgenus *Esula* were not susceptible.

The isolates evaluated to date generally are nonpathogenic to North American collections of leafy spurge and therefore should not be considered for introduction.

Table 2. Relative virulence of *Melampsora euphorbiae* isolate E-49 on Euphorbiaceae evaluated at ETH, Switzerland

Species	Origin		Plant reaction[a]
	Country	Locality	
Euphorbia esula	Italy	Passo Abetone	+++++[b]
E. esula	Italy	Mantova	+++
Leafy spurge[c]	Italy	Southern Tyrol	−
E. virgata	Germany	Heidelberg	++
E. virgata	Germany	Mainz	+
E. virgata	Germany	Dresden	+
E. virgata	Switzerland	Schaffhausen	+
E. virgata	Poland	Kaluszyn	+
E. esula	Poland	Warsaw	−
E. virgata	Poland	Warsaw	−
E. esula	France	Paris	−
E. esula	Canada	Saskatchewan	+
Leafy spurge[c]	Canada	British Columbia	+
E. esula	United States	Nebraska	+
Leafy spurge[c]	United States	Nebraska	−
E. esula	United States	Montana	−
E. esula	United States	Montana	−
Leafy spurge[c]	United States	Oregon	−
Leafy spurge[c]	United States	Idaho	−
Leafy spurge[c]	United States	California	−
E. esula	United States	Wyoming	−
E. cyparissias	Italy	Trieste	−
E. cyparissias	Switzerland	Bern	−
E. cyparissias	Switzerland	Zürich	−
E. lathyris	Switzerland	Zürich	−
Ricinus communis	Austria	Vienna	−

[a] − = No macroscopic symptoms; + = fewer than 10% of leaves infected, one or two sori per leaf; ++ = 10–35% of leaves infected, two to four sori per leaf; +++ = 35–65% of leaves infected, four to 10 sori per leaf; and +++++ = more than 90% of the leaves infected, more than 10 sori per leaf.
[b] Original host from which isolate was collected.
[c] Not identified to species.

Our experiences with *M. euphorbiae* from central Europe are similar to experiences with insect natural enemies from the same areas. Natural enemies from central Europe to date have not been well suited to spurges in North America. The evidence herein also supports the concept that leafy spurge in North America may differ from European material (6). Differences in susceptibility may be explained by the different geographic origins of host plants and pathogens on the hypothesis that most of the leafy spurge in North America came from Russia (4). Differences in susceptibility also may have developed during the process of adaptation of the spurges in North America, which includes the possibility of hybridization (16). It remains to be seen whether plant pathogens collected in southern Russia would be better suited to North American spurges.

Specificity of *M. euphorbiae* has been reported by Müller (12), who distinguished at least eight formae speciales of *M. euphorbiae*, each infecting only one or two species of *Euphorbia*. Determining the degree of specialization will require a better understanding of leafy spurge taxonomy in Europe and North America (including the relative abundance of hybrids) along with a study involving many strains of the fungus and collections of host plants. Such knowledge may lead to the use of this fungus for biological control of leafy spurge in North America either by matching spurge "types" with individual, virulent isolates of the fungus, by mixing two or more isolates of the fungus known to attack a range of leafy spurge "types," or through breeding and selection of fungal hybrids pathogenic to North American spurges.

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