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Effects of European and U.S. strains of *Fusarium* spp. pathogenic to leafy spurge on North American grasses and cultivated species[1]

A. J. CAESAR[2], G. CAMPOBASSO[3], and G. TERRAGITTI[3]

[2]Pest Management and Agricultural Systems Research Unit, Northern Plains Agricultural Research Laboratory, U.S. Department of Agriculture, Agricultural Research Service, Sidney, Montana 59270; and [3]USDA, ARS European Biological Control Laboratory, Rome Substation, Rome, Italy.

Abstract:

Host-range tests were conducted in a greenhouse using 9 European and 11 U.S. strains of *Fusarium* spp. pathogenic to *Euphorbia* spp. Plants of 12 grass species native to the rangelands of North America were raised from seed, planted in soil infested with each strain, and assessed for dry weight after 24 weeks. Five of 11 U.S. strains of *Fusarium* spp. significantly reduced the dry weight of at least 1 species of grass native to North America. Only 3 native grass species were affected: Idaho fescue (*Festuca idahoensis*), big bluestem (*Andropogon gerardi*), and big bluegrass (*Poa ampla*). Mean reductions ranged from 56 to 92%. A single European strain caused a 53% reduction in dry weight of 1 grass species, sand lovegrass (*Eragrostis tichodes*). Root-dip assays of 3-week-old seedlings in the greenhouse, with assessment over 3 weeks followed by 9 weeks of further observation and recording of dry weights of surviving plants were used to assess pathogenicity to 27 cultivated plant species. Two of the 3 most virulent U.S. strains failed to cause disease on any crop species according to these criteria. Three U.S. strains were positive in root-dip assays, each to a single crop species, causing vascular discoloration of flax (*Linum ussitatissimum*) and root necrosis of okra (*Hibiscus esculentus*) and cotton (*Gossypium hirsutum*). Neither of the 2 most virulent European strains exhibited pathogenicity to any of 12 crop species. Two other European strains exhibited host ranges comprised of 3 and 4 crop species. Two strains of *F. proliferatum* from the U.S. and Europe differing in host range were vegetatively compatible. The greater frequency of disease incidence on *Euphorbia* in Europe and the narrow host range and apparently greater

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virulence of European strains indicate that strains well-adapted to leafy spurge might best include *Fusarium* spp. occurring in Europe.

Keywords:

Biological control; mycoherbicide; rangeland weeds; *Euphorbia esula*, *Andropogon gerardi*, *Eragrostis tichodes*, *Festuca idahoensis*, *Poa ampla*.

Leafy spurge (*Euphorbia esula* L./*virgata* (L.) Waldst. & Kit.) is the most economically damaging perennial weed in the rangelands of the Northern Plains of the United States and the Prairie Provinces of Canada. It is an exotic weed with a native range that extends from western Europe to northeast China. Negative effects of the weed include reduced grazing capacity of rangelands due to toxicity to livestock and displacement or suppression of native range species, both leading to decreased land values. Economic losses are estimated to exceed $43.9 million (Bangsund and Leistritz, 1991), and there is the threat of increased infestation of croplands in the Northern Plains. *Fusarium* spp. have been isolated and described from diseased and dying leafy spurge plants found in natural stand-declines that occur rarely or in small patches in large stands of leafy spurge in the United States (Caesar, 1996). Diseased *Euphorbia* spp. are also found frequently in Europe (Caesar *et al.,* 1998), where the plant is widespread in small stands and is typically infested with one or more insects, including root-attacking species. Often the most virulent pathogen strains are associated with diseased roots fed upon by insects (Caesar, 1996; Caesar *et al.,* 1998). Strains of three *Fusarium* species, *F. oxysporum* Sclechtend.: Fr., *F. solani* (Mart) Sacc., and *F. proliferatum* (T. Matsushima) Nirenberg were highly virulent to leafy spurge in greenhouse tests (Caesar, 1996; Caesar *et al.,* 1998). Field applications of a formulated strain of *F. oxysporum* have reduced stand density by as much as 30% over a single season (Caesar, unpublished). Thus, such strains would be good candidates for integrated control of leafy spurge when applied as a formulated product. To assess the potential of strains of *Fusarium* spp. as biological control agents, whether collected in North America or from the larger body of strains associated with insect-damaged leafy spurge in its native range overseas, information is needed on the effects of the strains on nontarget plants. Species with the greatest potential to be affected by their application are range grasses native to North America. Therefore, the objective of this study was to assess the impact of *Fusarium* spp. on mature grass plants and the stage of the plant most likely to experience any immediate impact. Furthermore, it is necessary to assess the effects of these pathogens on cultivated plant species to determine the level of risk associated with their potential release into agroecosystems. As an initial step to elucidate relationships among U.S. and European strains of *Fusarium* spp. from leafy spurge, a further objective was to assess the vegetative compatibility between strains from both areas.

Materials and methods

Storage of *Fusarium* spp.

All strains were stored at -80°C in a nutrient broth (Difco) amended with 15% glycerol and at -20°C on sterile toothpicks or carnation leaves partially colonized by the strains.

Effects of *Fusarium* spp. on biomass of native grasses

The strains assessed in the present study have been described previously (Caesar, 1996; Caesar *et al*., 1998). The host ranges of the strains were assessed on 12 North American grass species listed in Table 1. European strains were tested in Rome, Italy and U.S. strains were tested in Bozeman, MT. The growth habits and seasonal characteristics of these grasses are also given in Table 1.

Table 1. North American native grasses used in this study.

Species	Common name	Height[a]	Season[b]	Growth habit
Agropyron dasystachyum (Hook) Lams.-Scribn.	Thickspike wheatgrass	M	C	Sod
Agropyron riparium Lams.-Scribn. and J. G. Sm.	Streambank wheatgrass	S-M	C	Sod
Andropogon gerardi Vitm.	Big Bluestem	T	W	Sod
Agropyron spicatum (Pursh.) Lams.-Scribn. & J. G. Sm.	Bluebunch wheatgrass	M	C	Bunch
Andropogon hallii Hack.	Sand bluestem	T	W	Sod
Bouteloua gracilis (H. B. K.) Lag. Ex Stend.	Blue grama	S-M	W	Bunch/Sod
Elymus canadensis L.	Canada wildrye	T	C	Bunch
Eragrostis tichodes (Nutt.) A. Wood	Sand lovegrass	T	W	Bunch
Festuca idahoensis Elmer	Idaho fescue	T	C	Bunch
Koeleria cristata Pers.	Prairie junegrass	M	C	Bunch
Poa ampla Merr.	Big bluegrass	T	C	Bunch
Schizachyrium scoparium (Michx.) Nash	Little bluestem	M	W	Bunch

[a] S, short (0.3-0.45 m maximum height); M, medium (0.45-1.1 m); T, tall (1.2-2.1 m).
[b] C, cool (April-June; Sept. 1-Nov. 1); W, warm (July 1 – Sept. 1).

Inocula for tests were prepared by culturing the strains in a 2% liquid medium in distilled water (w/v) consisting of Dietfiber (finely ground soybean hulls formerly manufactured by Lauhoff Grain Co., Danville, IL and containing ca 75% crude and soluble fiber, ca 10-12% protein, ca 1% fat, ca 5% ash, and ca 4% moisture, all w/w). The cultures were grown at 25°C for 2-3 weeks with shaking and thoroughly mixed with a pasteurized greenhouse potting medium to achieve ca 150 colony-forming units (cfu) per g of air-

dried soil mix. Populations of *Fusarium* were determined by plating four-fold dilutions of soil on Nash and Snyder medium (Nash and Snyder, 1962) and processing the data to obtain the most probable number of cfu (Clark and Owens, 1983).

In pathogenicity tests conducted with U.S. strains, the potting medium was a 1:1:1 v/v/v Bozeman silt loam, peat, and sand, pH 6.6, mixture. In tests conducted in Rome, the potting medium was a commercially sold mixture of 40% organic compost, 10% sand, 20% sphagnum peat moss and 30% loam soil. The grass seeds were sown in 2.0-cm-diameter Cone-tainers (Stuewe and Sons, Inc., Corvallis, OR), thinned to two seedlings per cell, and grown at 20-28°C in the greenhouse. Eight- to 10-week-old plants were removed from 2-cm-diameter Cone-tainers and transplanted into 4-cm-diameter Cone-tainers of the infested soil. A treatment consisted of five Cone-tainers of soil mix infested with a strain of *Fusarium* and planted with a plug of a single grass species. Controls consisted of grass planted in 4-cm-diameter Cone-tainers containing uninfested soil. The grasses were grown at 20-28°C in the greenhouse, supplied with water every other day and Hoagland's solution weekly, and harvested 24 weeks after transplanting into infested soil. At harvest, grass plants were removed from Cone-tainers, washed free of the soil mix, dried at 28°C, and weighed. After weighing, samples of root and crown tissues were plated on water agar. The tests were repeated twice. Data were tested to confirm homogeneity of variances by the method of Bartlett and Kendall (1946) prior to pooling data from all trials for analysis using Waller and Duncan's exact Bayesian k-ratio LSD rule ($P = 0.05$) (Waller and Duncan, 1969).

Tests of pathogenicity on cultivated species

Three-week-old seedlings of 27 cultivated species were subjected to pathogenicity tests of each of the U.S. strains of *Fusarium* spp. using root-dip assays (Elmer, 1991; Katan *et al*., 1994; MacDonald and Leach, 1976; Rowe, 1980; Salgado and Schwartz, 1995; Swanson and Van Gundy, 1985). Testing of U.S. strains was conducted in Bozeman, MT. Testing of European strains on 12 cultivated species was conducted in Rome, Italy. Seed of various species were planted in the appropriate artificial potting mix described in the previous section in 24 to 48-cell plastic planting trays consisting of 5-to 6-cm-square cells or sections (liners). Thinning 10 days after emergence resulted in 2 seedlings per cell. Inocula for root-dip assays were prepared as liquid cultures of 2% (w/v) Dietfiber at 20-28°C with shaking and filtered through two layers of cheesecloth. Suspensions consisting of microconidia, macroconidia, and chlamydospores (*F. oxysporum* and *F. solani*) or macroconidia and microconidia (*F. proliferatum*) were prepared at 10^6 cfu/ml using a hemacytometer to estimate spore concentrations. Roots of 3-week-old seedlings of various species were removed from trays, carefully washed free of soil, and dipped for 3-5 min in spore suspensions prior to planting. Five seedlings per strain were dipped and planted in 7.5-cm-diameter pots completely randomized by treatment. Species inoculated were alfalfa (*Medicago sativa* L.) cv Washoe, artichoke (*Cynara cardunculus* L.) cv Green Globe, asparagus (*Asparagus officianalis* L.) cv Mary Washington, bachelors button (*Centaurea* cyanus L.) cv Finest Mixed, barley (*Hordeum vulgare* L.), field corn (*Zea mays* L.), carnation (*Dianthus caryophyllus* L.) cv Chabaud, sweet corn (*Zea mays* L.) cv Golden Bantam, cotton (*Gossypium hirsutum* L.) cv Stoneville 7A, cowpea (*Vigna unguiculata* ((L.) Walp.) cv California Blackeye, flax (*Linum ussitatissimum* L.) cultivar

Punjab, mung bean (*Phaseolus aureus* L.-*Vigna radiata* (L.) R. Wilcz.) cv Berken, muskmelon (*Cucumis melo* L. var. *reticulatus*) cv Earliqueen, oats (*Avena sativa* L.), okra (*Hibiscus esculentus* L.) cv Clemson Spineless, peanut (*Arachis hypogea* L.) cv Virginia Jumbo, rice (*Oryza sativa* L.), rye (*Secale cereale* L.), safflower (*Carthamus tinctorus* L.), snapbean (*Phaseolus vulgaris* L.) cv Blue Lake 274, sorghum (*Sorghum bicolor* (L.) Moench), soybean (*Glycine max* (L.) Merr.) cultivar Essex, sugarbeet (*Beta vulgaris* L.), garden beet (*Beta vulgaris* L.) cv Detroit Red, sunflower (*Helianthus anuus* L.) cv D131, (*Lycopersicon esculentum* Miller) cv Bonny Best, wheat (*Triticum aestivum* L.), and zinnia (*Zinnia violacea* Cav.) cv State Fair Mixed. In tests conducted in Rome, 3-week-old seedlings of asparagus, bachelors button, carnation, corn, flax, muskmelon, okra, snapbean, tomato, and zinnia, each of the same cultivar as above, were used. Inoculated seedlings were planted into soil mix in liners or 4-cm-diameter Cone-tainers, grown at 20-25°C, and observed for the symptoms described in previous studies using the root-dip assay to test pathogenicity on various species. Respective controls for all tests consisted of seedlings dipped in water prior to replanting in pasteurized soil mix. If seedling mortality or chlorosis was observed within 3 weeks after tests began, the test was scored as positive. The test plants were then left to grow for another 6 weeks in the greenhouse for further observation. If chlorosis or apparent stunting of any replicate was observed relative to the control for any plant species, all plants of the species were harvested, dried at 28°C, and weighed. Samples of root and crown tissue of weighed plants were plated onto water agar to detect the presence of the respective inoculated strain. These series of tests were repeated twice. Data were tested to confirm homogeneity of variances by the method of Bartlett and Kendall (1946) prior to pooling data from all trials for analysis using Waller and Duncan's exact Bayesian k-ratio LSD rule ($P = 0.05$) (Waller and Duncan, 1969).

Vegetative compatibility group testing of *Fusarium oxysporum* strains

Several defined nitrogen source media (FDNS) were prepared by amending a minimal medium (MM) containing 3% sucrose and 0.17% yeast nitrogen base without amino acids or ammonium sulfate (Difco) (Pulhalla, 1985; V. Miller, personal communication) with one of several different nitrogen sources (Correll *et al*., 1987). The MM amended with 5% sodium chlorate (YC) was used to generate nitrate nonutilizing mutants (*nit*) (Pulhalla, 1985). FDNS were used to characterize phenotypically nit mutants and the nitrate FDNS was used to recognize *nit* mutants and for complementation (heterokaryon) tests. *Nit* mutants were generated by placing small mycelial plugs of *Fusarium* strains on YC medium and observing for fast growing sectors over 4-10 days. The *nit* mutants were then grown on FDNS to phenotypically characterize the mutants. Three different *nit* mutants, *nit*1*, nit3,* and *nit*M were obtained from all four *F. oxysporum* strains and the single strain of *F. proliferatum.* Some *nit* mutants were obtained from two of the four strains of *F. solani:* two *nit* mutants, *nit*1 and *nit*M*,* were generated from 94f-15 and a single *nit*M mutant was generated for 94f-24. U.S. *nit* mutants were paired in all possible combinations with European *nit* mutants on nitrate FDNS, and pairings were scored as vegetatively compatible when wild-type mycelial growth occurred within 2 weeks at colony interfaces between mutant strains. All complementation tests were made at least twice.

Results

Effects of European and U.S. strains on native grasses

One or more U.S. strains of *Fusarium* spp. pathogenic to leafy spurge caused significant reductions in dry weight of 3 of 12 species of North American grasses: Idaho fescue, big bluestem, and big bluegrass, 24 weeks after the grasses were planted in soil infested with the various strains. In no instance was seed-head production affected. Seed was produced by all grasses, although total seed production was not measured. Reductions in dry weight of Idaho fescue were caused by strains Lyman Creek 4A, Sidney 3Y, MT94-20,

Table 2. Results of host-range tests of *Fusarium* spp. from the U.S. northern plains on 27 cultivated species and 12 native grasses.

Strain and U.S. state of origin[a]	Species	Virulence Ranking[b]	Cultivated species with significant reductions in dry weight and percentage of reduction in brackets[c,f]	Cultivated species with reaction to root-dip assay and symptoms[d]	North American native grasses with significant reductions in dry weight and percentage of reduction in brackets[e,f]
ND94-8 North Dakota	*Fusarium oxysporum*	1	None	Flax (chlorosis and wilt)	None
Lyman Creek 4A Montana	*F. oxysporum*	2	None	None	Idaho fescue (58%) Big bluestem (87%) Big bluegrass (71%)
Fairy Soil 3B Montana	*F. oxysporum*	3	None	None	Big bluestem (83%)
McLain 2 Montana	*F. oxysporum*	4	Sorghum (65%) Sugarbeet (71%) Cantaloupe (80%)	Okra (seedling mortality)	None
Sidney 3Y Montana	*F. proliferatum*	5	None	Cotton (seedling mortality)	Idaho fescue (57%) Big bluestem (92%) Big bluegrass (56%)
Fairy Soil 3V Montana	*F. solani*	6	None	None	None
ND94-10 North Dakota	*F. oxysporum*	7	None	None	Big bluestem (64%)
MT94-20 Montana	*F. oxysporum*	8	None	None	Idaho fescue (60%)
ND94-5 North Dakota	*F. oxysporum*	9	None	None	None
ND94-6a North Dakota	*F. solani*	10	Safflower (72%)	None	None
WY94-11-4 Wyoming	*F. oxysporum*	11	None	None	Idaho fescue (62%) Big bluestem (77%)

[a] Detailed collection locations, collection dates, symptomatology on original host, and association with insect damage have been previously described (Caesar, 1996).
[b] Based on 0-6 rating scale of disease of leafy spurge planted in soil infested with equal populations of *Fusarium* spp. (Caesar, 1996).
[c] Cultivated species (of 27 tested) which exhibited reduced dry weight within 9 weeks after transplanting into greenhouse soil mix following root-dip assays using spore concentrations of 10^6 spores/ml. Expressed as a percentage compared to the untreated control. See Materials and Methods for a list of cultivated species screened in host range tests.
[d] Cultivated species (of 27 tested) which exhibited mortality or symptoms of vascular wilt, compared to the untreated control (water dip) in greenhouse tests within 3 weeks following root-dip assays using a spore concentration of 10^6 spores/ml.
[e] Significant reductions in dry weight ($P = 0.05$) 9 weeks after planting into greenhouse soil mix infested with *Fusarium* spp. At 150 cfu/g of air-dried soil mix. See Table 1 for a list of North American native grass species tested.
[f] Significant differences were determined using ANOVA followed by Waller and Duncan's exact Bayesian k-ratio LSD rule.

and WY94-11-4 and ranged from 57 to 62% (Table 2). Dry weights of big bluestem were significantly reduced by five strains, Lyman Creek 4A, Fairy Soil 3B, Sidney 3Y, ND94-10, and WY94-11-4, and ranged from 64 to 92%. Two strains, Lyman Creek 4A and Sidney 3Y, significantly reduced dry weight of big bluegrass by 71 and 56%, respectively. A strain, ND94-8, highly virulent in a previous study, failed to affect any grass negatively. Similarly, strains McLain 2, Fairy Soil 3V, ND94-5, and ND94-6a failed to have any effect on native grasses. Only a single European strain of *F. solani* tested, 94f-24, significantly reduced the biomas of any of the 12 North American grasses in tests overseas over 24 weeks (Table 3).

Table 3. Results of host-range tests of *Fusarium* spp. from Europe on 12 cultivated species and 12 native grasses.

Strain and country of origin[a]	Species	Virulence ranking vs spurge[b]	Cultivated species[c] with significant reductions in dry weight and percentage of reduction in brackets[f]	Cultivated species with reaction to root-dip assay and symptoms[d]	North American native grasses with significant reductions in dry weight and percentage of reduction in brackets[e,f]
94f-5 Russia	*Fusarium oxysporum*	1	None	None	None
94f-37 France	*F. oxysporum*	2	None	None	None
94f-25 Russia	*F. solani*	3	Muskmelon (100%)	None	None
			Zinnia (100%)	None	None
			Snapbean (100%)	None	None
94f-24 Russia	*F. solani*	4	None	None	Sand lovegrass (53%)
94f-11 Russia	*F. oxysporum*	5	Okra (100%)	None	None
			Muskmelon (88%)	None	None
			Zinnia (100%)	None	None
			Snapbean (73%)	None	None
94f-15 Russia	*F. solani*	6	Snapbean (100%)	None	None
			Zinnia (98%)	None	None
93f-17D Russia	*F. oxysporum*	7	Snapbean (100%)	None	None
94f-29 Russia	*F. solani*	8	None	None	None
94-30 Russia	*F. proliferatum*	9	None	None	None
Control		–	–	–	–

[a] Detailed collection locations, collection dates, symptomatology on original host and association with insect damage have been previously described (Caesar *et al*., 1998).
[b] Based on 0-6 rating scale of disease of leafy spurge planted in soil infested with equal populations of *Fusarium* spp. (Caesar *et al*., 1998).
[c] Cultivated species (of 12 tested) which exhibited reduced dry weight within 9 weeks after transplanting into greenhouse soil mix following root-dip assays using spore concentrations of 10^6 spores/ml. Expressed as a percentage compared to untreated control. Cultivated species included in this host range are listed under Materials and Methods.
[d] Cultivated species (of 12 tested) which exhibited mortality or symptoms of vascular wilt, compared to the untreated control (water dip), in greenhouse tests within 3 weeks following root-dip assays using a spore concentration of 10^6 spores/ml.
[e] Significant reductions in dry weight ($P = 0.05$) 9 weeks after planting into greenhouse soil mix infested with 150 cfu/g of air-dried soil mix of *Fusarium* spp. See Table 1 for a list of North American native grass species tested.
[f] Significant differences were determined using ANOVA followed by Waller and Duncan's exact Bayesian k-ratio LSD rule.

Effects of U.S. strains on 27 cultivated species

Using root-dip assays, cotton and okra were found to exhibit seedling mortality due to McLain 2 and Sidney 3Y, respectively (Table 2). Flax exhibited chlorosis and wilt within 3 weeks after the assay, caused by ND94-8, although this was not reflected in a reduction in dry weight over a total of 9 weeks of growth. McLain 2 caused significant reductions ($P = 0.05$) in dry weight of sorghum, sugarbeet, and cantaloupe, of 65, 71, and 80%, respectively. Strains of *Fusarium* spp. reduced dry weights of only 4 (cantaloupe, sorghum, sugarbeet, and safflower) of 27 cultivated species when plants from root-dip assays were allowed to grow for 9 weeks after planting. However, a single strain of *F. oxysporum*, McLain 2, caused three instances of such dryweight reductions.

Effects of European strains on 12 cultivated species

Neither of the two most virulent European strains, 94f-5 and 94f-37, both previously identified as *F. oxysporum* (Caesar *et al*., 1998), caused apparent disease or reduced dry weight of any of 12 cultivated species on which they were tested (Table 3). No European strain caused disease within 3 weeks after inoculation by root-dip. Four European strains, two identified as *F. solani*, 94f-25 and 94f-15, and two as *F. oxysporum*, 94f-11 and 93f-17D, caused mortality to 1 or more cultivated species within 9 weeks but after 3 weeks following root-dip assays. Cultivated species which exhibited complete mortality in all replications between 3 and 9 weeks after root-dips were muskmelon, zinnia, snapbean, and okra. The widest range of hosts attacked by the four strains was 4 species, exhibited by 94f-11, with the three other strains, 94f-25, 94f-15, and 93f-17D, exhibiting host ranges of 3, 2, and 1 species, respectively.

Vegetative compatibility tests

Two strains of *F. proliferatum*, one from Sidney, MT (Sidney 3Y), the other from Tatarka, Russia (94f-30) were vegetatively compatible. The Russian strain was isolated from dead root adventitious buds of *Euphorbia virgata* and was ranked lowest in virulence among nine strains from Europe described in a previous study (Caesar *et al*., 1998). No other pairing of a European and a U.S. strain resulted in vegetative compatibility.

Discussion

The widest range of hosts attacked by any U.S. strain of *Fusarium* spp. on 12 North American grasses was three, exhibited by two strains, Sidney 3Y and Lyman Creek 4A. Only one European strain, 94f-24, significantly reduced the dry weight of any of the 12 North American grasses. The widest range of hosts attacked by any U.S. strain when tested on 27 cultivated species was three. The widest range of hosts of any European strain on 12 cultivated species was four, exhibited by one strain, 94f-11. Three of the five most virulent U.S. strains of *Fusarium* spp., based on a previous study (Caesar, 1996), exhibited the widest host ranges against either North American grasses or cultivated species. However, of the four most virulent European strains, only one, 94f-25, was patho-

genic to more than 1 cultivated species or North American grass. Additionally, neither of the two European strains most virulent to leafy spurge, among nine assessed, caused disease or reduced the biomass of any of 12 cultivated crop and 12 North American grass species tested. A critical criterion for the potential utility of *Fusarium* spp. as a biological control agent of a target weed is the host range, particularly among North American grasses. Such grasses are the nontarget plants with the greatest and most immediate chance of being impacted should any of the *Fusarium* spp. be applied to stands of leafy spurge for biological control. These collective results are promising and justify wider search and screening of strains with high virulence and narrow host ranges. These results also provide preliminary indication that searches beyond North America could potentially lead to safer strains for use as biocontrol agents of weeds.

The need for more extensive search and screening is further supported by existing knowledge of host specificity among *Fusarium* spp. in general. Formae speciales of *F. oxysporum* and *F. solani*, especially wilt-causing *F. oxysporum*, often exhibit pronounced host specificity (Booth, 1971). For example, formae speciales that are restricted in host range to a single species (Snyder, 1941) or to subspecific groups within a single species (Armstrong and Armstrong, 1976) have been identified. An alternative model of pathogenicity and host range would be that of *F. oxysporum* f. sp. *Radicislycopersici* (Rowe, 1980), a forma specialis consisting of root- and crown-rot pathogens that are highly host specific (Jarvis *et al*., 1975) or typically have a wider host range while exhibiting greater virulence to the host of origin (Rowe, 1980). Based on the symptomatology for these European strains, as mostly root- and crown-rot pathogens (Caesar *et al*., 1998), and the host ranges described herein, they may more closely resemble the former pattern for strains of *F. oxysporum* f. sp. *radicis-lycopersici*. The U.S. strains seem to be more similar to wilt *Fusaria*, based on a previous study (Caesar, 1996). Testing of U.S. strains isolated since an earlier study has supported this hypothesis (Caesar, unpublished). Studies of the host range of *Fusarium* spp. within the two subgeneric taxa that define the closest wild and endangered relatives of *Euphorbia esula* (Pemberton, 1985) are needed.

One U.S. and 1 European strain of *F. proliferatum* were vegetatively compatible. Of the 11 strains compared in a previous study (Caesar, 1996), the U.S. strain was moderately ranked in virulence, whereas the European strain was of comparatively low virulence among the 9 strains compared in another study (Caesar *et al*., 1998). This finding confirms earlier research (Jacobson and Gordon, 1990; Elmer, 1991) showing that virulence may vary within a single vegetative compatibility group (VCG). Apparently there is also variation in host range within a single VCG, based on the present findings that the U.S. strain of *F. proliferatum* severely affected three grass species native to North America and one cultivated species, whereas the European strain failed to affect any species.

Diseased leafy spurge plants are more widespread and ubiquitous in Eurasia than in North America and occur within small, nondescript stands along roadsides and in natural areas. Thus, there is a greater pool of diseased spurge from which desirable strains may be isolated, tested, and selected. The frequency with which highly virulent, narrow host-range strains of *Fusarium* were found, as indicated by the present data and a previous study on European *Fusarium* spp. (Caesar *et al*., 1998), additionally shows that surveys beyond North America are promising. Evidence for these *Fusarium* strains and for other formae speciales indicates that searches for narrow host-range/high-virulence

strains in Eurasia may not necessarily result in strains highly exotic to North America since *Fusarium* spp. are cosmopolitan and ubiquitous. The evidence includes the similarity in the range of pathogen species associated with diseased or insect-damaged roots of leafy spurge in the U.S. and Europe, respectively, and the vegetative compatibility of U.S. and European strains of at least one species, *F. proliferatum*. In addition, no wide divergence in the host ranges was observed between foreign and domestic strains of any of three *Fusarium* spp. pathogenic to leafy spurge. Finally, the finding that the two most virulent European strains were the two strains with the narrowest host ranges among all U.S. and European strains assessed strengthens this view.

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