Fusarium Root Rot of Forage Species: Pathogenicity and Host Range

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ABSTRACT

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The pathogenicity of *Fusarium roseum*, *F. oxysporum*, *F. solani*, and *F. moniliforme*, isolated from diseased roots of forage plants, was compared on eight forage species in nutrient solution culture. The fungi were grown on polyester cloth strips placed on V-8 juice agar, and these strips were used to inoculate individual roots. The length of rot, frequency of infection, and inhibition of root growth were used to rate pathogenicity. Root tips were more susceptible to attack by the *Fusarium* spp. than were areas 2 cm above the root tip. Some isolates of *F. roseum* did not cause rot symptoms except at the root tip. Roots inoculated at the root tip often ceased elongation before they were penetrated by the fungus. White clover and birdsfoot trefoil were the most susceptible to root rot caused by *Fusarium* spp.; orchardgrass and crownvetch were the most resistant; and alfalfa, red clover, subterranean clover, and *Coronilla globosa* were intermediate in susceptibility. Isolates of the four *Fusarium* spp. usually gave greater frequency of infection, linear extent of rot lesions, and inhibition of root growth on the host species from which they were originally isolated than on the other species tested. Stress treatments such as clipping, foliar disease, and darkness, reduced root growth but did not affect root rot development in red clover. The relative pathogenicity of isolates of *F. roseum* to plants grown in nutrient solution culture was confirmed by inoculations of severed taproots of 4-mo-old red clover and alfalfa plants in soil in the greenhouse.

Additional key words: inoculation methods, *Medicago sativa*, *Coronilla varia*, *Trifolium pratense*, *Trifolium repens*, *Trifolium subterraneum*, *Dactylis glomerata*, *Lotus corniculatus*.

Fusarium causes root and crown rot of forage legumes in most production areas (3, 5, 9, 10), and is of major importance in the northeastern United States (9). Several species and many strains of *Fusarium* have been associated with this disease, but their importance as causal agents has not been defined, because they are part of a complex involving other organisms and various stress factors. Many questions remain concerning the ability of these *Fusarium* spp. to penetrate roots directly, their degree of host specificity, and their interaction with plant stress factors. Some *Fusarium* isolates associated with root rots of forages penetrate roots directly (1, 4). Wounding of roots significantly increases colonization by other isolates (6, 15). There also is evidence to both support (2) and refute (1) the presence of host specificity in this group of fungi.

The slant-board method of growing plants (7) provided the means to investigate some of these relationships. This is a nutrient solution culture method in which the roots grow between layers of polyester cloth, thus affording frequent, noninjurious access to the roots, replicated inoculation of a single plant, and a means to determine the degree of rot and its effect on plant growth (8).

The objectives of this research were to compare the pathogenicity of various isolates of *Fusarium* from diseased forage roots to determine the relationship between root rot severity and root growth; to evaluate the effects of various plant stress factors on the development of root rot; to compare the relative susceptibility of different root sites to infection; to compare forage species for susceptibility to root rot; and to study the degree of host specificity present in *Fusarium* associated with root rots. Our approach assessed the full pathogenic potential of these fungi under conditions that afforded direct contact between the root and the fungus in the absence of modifying influences of rhizosphere and soil microorganisms.

MATERIALS AND METHODS

Roots of single plants, about 1 mo old and grown on individual slant-boards (7), were spread fan-like (Fig. 1-A), and 10 or more roots per plant were inoculated. Although no attempt was made to keep the slant-board cultures sterile, the following procedures were used to minimize contamination by *Thielaviopsis basicola* which thrives in the slant-board system: cloths and bags were washed and autoclaved before use; trays were washed thoroughly before use; cloths were changed frequently before inoculation; seeds were surface-disinfested and germinated in Perlite; and plants were evaluated within 4 days after inoculation. Periodically, nutrient solution draining from slant-boards was assayed on several culture

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media to detect microbial contaminants. Usually *Alternaria*, *Penicillium*, a nonsporulating yellow fungus, a pink yeast, and some bacteria occurred at low levels. Root tissue inoculated with *Fusarium* but not subjected to surface disinfestation yielded predominately *Fusarium* with occasional isolations of *Rhizopus* and *Penicillium*.

All isolates of *Fusarium* originally came from diseased plant tissue and were stored in dry soil at 4 C. Identifications were verified by the Fusarium Research Center at The Pennsylvania State University. Inoculum was started by placing infested soil in V-8 juice agar (11) and incubating plates at 20-22 C exposed to cool-white fluorescent light at 500 lux for 12 hr daily. The fungus overgrew 1- × 1.5-cm sterile strips of polyester cloth (the same cloth used in the slant boards) laid on the surface of the agar. These strips, covered with fungus, were lifted from the agar surface after 10 days and used to inoculate individual roots in slant boards (Fig. 1-A). No culture substrate adhered to the polyester cloth strips. Inoculum consisted of mycelia and conidia; no chlamydospores formed on the inoculum strips before inoculation. Fungal inoculum strips were positioned to cover segments of roots located either 0 to 1 cm or 2 to 3 cm above the root tip. These are referred to as the tip and 2-cm inoculation sites.

The first experiments consisted of screening 51 *Fusarium* isolates for pathogenicity against red clover and alfalfa roots. The isolates included members of *F. moniliforme* (Sheld.) emend Snyd. & Hans., *F. oxysporum* (Schl.) emend Snyd. & Hans., *F. roseum* (Lk.) emend Snyd. & Hans., *F. solani* (Mart.) Appel & Wr. emend Snyd. & Hans., and *F. tricinctum* [Cda.] emend Snyd. & Hans. Each isolate was categorized for its ability to attack root tips and at 2 cm above the root tip. Such screening resulted in the selection of a group of isolates that exhibited a range in pathogenicity and produced consistent inoculations.

A test set of four isolates of *F. roseum* was used to inoculate roots of various cultivars of host species. The isolates and their origins were Avenaceum (766) from red clover, Accuminatum (927) from alfalfa, Accuminatum (959) from red clover, and Accuminatum (1055) from *Coronilla globosa*. The cultivars and species inoculated with the isolates were: 'Pennscott', 'Kenland', and

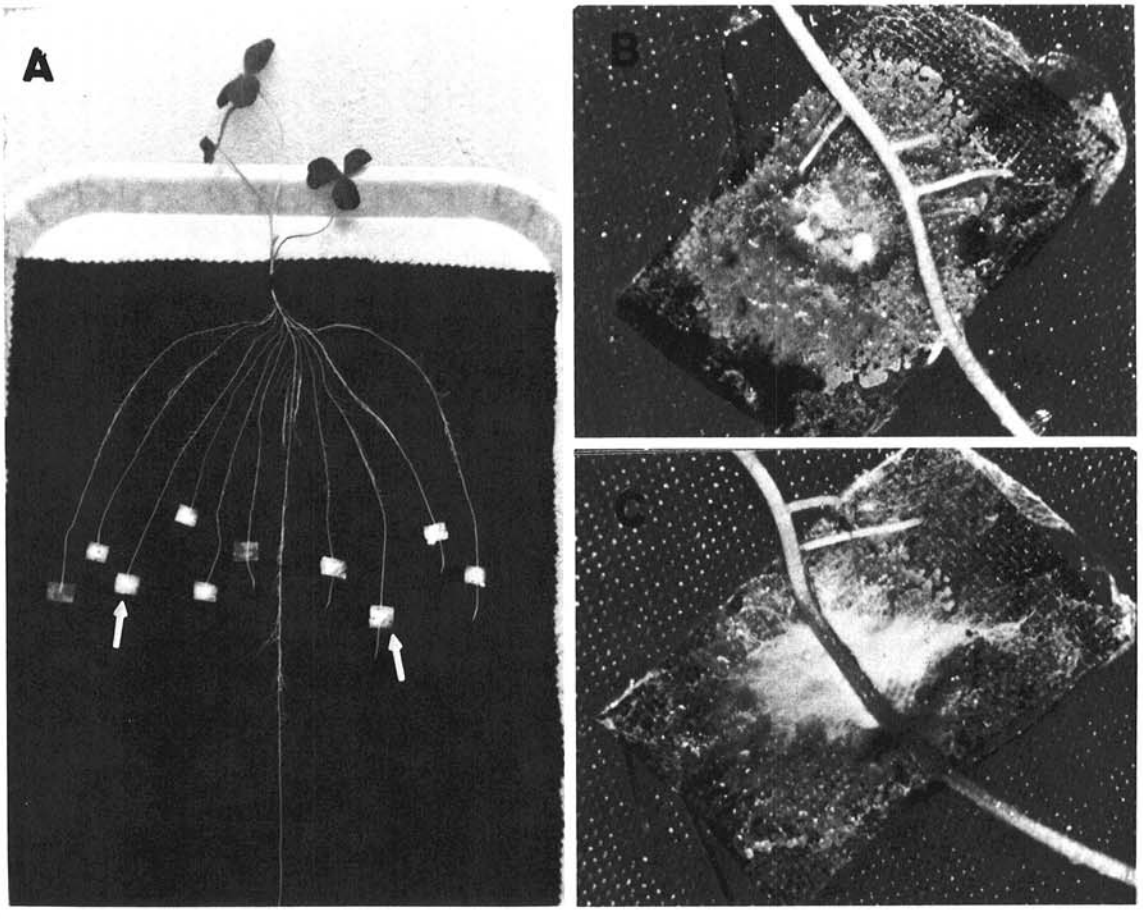

Fig. 1-(A to C). Illustration of the method of inoculation and some typical reactions of roots to inoculation with *Fusarium roseum*. **A)** Four-wk-old red clover plant with roots spread fan-like on a slant-board surface. Arrows point to inoculum strips applied at the root-tip and at 2-cm above the root tip. **B)** Symptomless root in contact with nonpathogenic isolate of *F. roseum* 'Avenaceum' (× 3.5). **C)** Typical rot caused by isolate of *F. roseum* 'Acuminatum' (× 3.5).

'Lakeland' red clover (*Trifolium pratense* L.); 'Du Puits', 'Saranac', 'Apalachee', 'Lahontan', and plant introduction (P.I. 233715) alfalfa (*Medicago sativa* L.); four clones of 'Penngift' orchardgrass (*Dactylis glomerata* L.); 'Dawn' birdsfoot trefoil (*Lotus corniculatus* L.); and 'Chemung' crownvetch (*Coronilla varia* L.). All plants were grown from seed except the orchardgrass, which was propagated vegetatively with ramets.

The host range of 21 *Fusarium* isolates was investigated by cross-inoculating the eight forage species from which they were isolated with each fungus. All inoculations were made at the root tips. Data recorded were frequency of rot, length of rot lesion, number of forage species attacked, and root growth inhibition. Forage species included in this experiment were: Arc alfalfa, a breeding line of *C. globosum* L., Emerald crownvetch, ladino white clover (*T. repens* L.), Pennscott red clover, Penngift orchardgrass, and Mississippi ecotype of subclover (*T. subterraneum* L.).

The slant-board method of growing plants was a good system for evaluating the effect of plant stress on the development of Fusarium root rot, because it was possible to determine accurately how severely the stress had affected root elongation at the time of inoculation. The following stresses were imposed on Kenland red clover: Stemphylium leafspot for 11 days, darkness for 10 days, or defoliation. Inoculations were made at the 2-cm site with *F. roseum* isolates 927 and 1055.

All experiments described thus far were done in a controlled-environment chamber with a 14-hr daily photoperiod, a light intensity from cool-white fluorescent and incandescent lamps of about 32,000 lux, and temperatures of 25 ± 1 and 15 ± 1 C during the light and dark periods, respectively.

Observations usually were made 3 or 4 days after inoculation. Rot, as determined by brown discoloration at inoculation site, was measured with a ruler and presented as length of rot; root elongation was measured with a ruler from the root tip position at time of inoculation and expressed in centimeters of growth or percent inhibition compared to elongation of control roots. Infection frequency percent was calculated by dividing the number of roots inoculated into the number of rots observed. The experimental design varied somewhat depending upon the number of factors involved in an experiment. Usually two noninoculated control roots were maintained on each plant, two roots per plant inoculated with each isolate, and seven to nine roots were inoculated per isolate per inoculation site in each experiment. Statistical significance of differences was determined by analysis of variance of a completely randomized design at $P = 0.05$.

Selected root pieces inoculated with *Fusarium* were cleared in chloral hydrate, stained with cotton blue, and examined at ×100 magnification for internal hyphae.

To determine if the pathogenicity expressed by the test set of *Fusarium* isolates on young roots of plants grown on slant boards was typical of their relationship with roots of more mature plants in soil, we inoculated severed taproots of 4-mo-old Pennscott red clover and Saranac alfalfa plants grown in a peat-soil mix in the greenhouse. Inoculation was made by removing the soil-root mass from its pot, cutting completely through the sod 4 cm below the soil surface, placing a fungal inoculum strip against the cut end of the taproot, and reassembling and repotting the sod. After 3 wk of incubation in the greenhouse for red clover and 4 wk for alfalfa, the root was split longitudinally above the inoculation site, and the amount of vertical discoloration was measured with a ruler.

TABLE 1. Length of rot[a] and elongation of roots of Pennscott red clover 4 days after inoculation at two sites with the test set of isolates of *Fusarium roseum*

Cultivar and isolate no.	Tip inoculation[b]		2-cm inoculation[c]	
	Rot (cm)	Root (cm)	Rot (cm)	Root (cm)
None (control)	0.0 A[d]	7.2 A	0.0 A	7.0 A
Avenaceum 766	0.0 A	6.0 A	0.0 A	7.0 A
Acuminatum 927	0.8 B	1.5 B	0.0 A	6.9 A
Acuminatum 1055	1.2 B	0.4 B	0.2 A	6.6 A
Acuminatum 959	1.5 B	0.0 B	2.0 B	1.2 B

[a]Length of rot is the length of root rotted after exposure for 4 days to fungal mycelium cultured on a 1.0 × 1.5-cm rectangle of polyester cloth lying on V-8 agar. The roots were attached to growing plants and maintained between layers of polyester cloth kept saturated with nutrient solution.

[b]Inoculum placed on tip of root.

[c]Inoculum placed on root 2 cm above the tip.

[d]Values followed by different letters are significantly different at $P = 0.05$ using Duncan's new multiple range test.

RESULTS

From the screening of our collection of *Fusarium* isolates, we selected four isolates of *F. roseum* that had good stability in culture, represented a range in pathogenicity on both red clover and alfalfa, and produced consistent disease reactions in the slant-board culture system. Isolate 766 was chosen because it did not cause rot or affect root growth (Fig. 1-B); isolate 927, because its pathogenicity was limited to the root tip; isolate 1055, because it attacked roots consistently at the tips and about 50% of the time at the 2-cm inoculation site as well; and isolate 959 because it was the most virulent of all the isolates at either inoculation site (Fig. 1-C).

On red clover, isolate 766 caused no rot, nor did it affect root elongation (Table 1); isolate 927 caused rot and inhibited root elongation when used to inoculate root tips but was ineffective at the 2-cm inoculation site; isolate 1055 caused rot at both inoculation sites, but rot was greater at the root-tip site, and inhibition of root elongation occurred only when root tips were inoculated; isolate 959 caused rot at both inoculation sites, complete inhibition of root elongation at the root-tip site, and significant inhibition of root elongation even when inoculation was made 2 cm above the root tip. Microscopic examination of cleared, stained roots revealed internal hyphae with all isolates of *Fusarium*, except 766, following root tip inoculations. Also, internal hyphae were always observed following inoculation at the 2-cm site with isolate 959, most of the time with 1055, but never with isolate 927. Chlamydospores usually were present on and in roots showing rot symptoms, and

sometimes present when rot symptoms were not evident.

On the basis of length of rot and elongation of roots of alfalfa after inoculation with the isolates of *F. roseum*, isolate 766 had no pathogenic activity, and isolates 927 and 1055 caused rot only with the root-tip inoculations, which also inhibited root elongations (Table 2). These isolates caused no rot at the 2-cm inoculation site. Isolate 959 caused rot at both inoculation sites, with more rot at the tip and inhibition of root elongation only after tip inoculation.

Often no elongation occurred with root-tip inoculations, which indicated that root growth was inhibited as soon as the tip contacted the fungus. When inoculum strips were removed after 6 or 12 hr of contact with root tips infection occurred by 12 but not by 6 hr of contact between roots and inoculum. Thus, root growth was inhibited by some isolates before the roots were infected, Established rots expanded at about 1.1 mm per day from root-tip inoculations and at slightly faster rates from the 2-cm inoculations, because expansion was both up and down the root. Longer rots were required at the 2-cm site to cause the same degree of root growth inhibition as that caused by the root-tip inoculations.

The behavior of the test set of isolates of *F. roseum* was similar on the three cultivars of red clover and on the three cultivars and the plant introduction of alfalfa, that is, isolate 766 caused no rot on any roots; isolate 927 attacked root tips; isolate 1055 attacked root tips and caused some rot at the 2-cm sites; isolate 959 attacked both tip and 2-cm sites. The total number of plants inoculated was too small to permit accurate comparison of cultivars.

Rot caused by *F. roseum* on orchardgrass roots also was comparable to that caused on red clover roots. Isolate 766 caused no rot; the other isolates ranked according to increasing virulence were 927, 1055, and 959. Root-tip inoculations with 927 and 1055 rapidly inhibited root elongation but 959, even though it caused severe rot, did not inhibit root elongation as quickly.

On birdsfoot trefoil isolate 766 caused no rot; the other isolates ranked according to increasing virulence were 1055, 927, and 959. Root elongation was inhibited by the three isolates that caused rot. Overall, the pathogenicity of the test set of isolates on birdsfoot trefoil was about the same as on red clover.

The isolates caused less rot on crownvetch than on any other host. Isolate 766 caused no rot and the other isolates ranked according to increased virulence were 927, 1055, and 959. Root elongation was inhibited by all isolates that caused rot at the root-tip site of inoculation.

The results of our host-range test are summarized in Table 3. The mean infection frequency of isolates on their original hosts was 30% compared with only 6% on the other host species. The mean length of rot on original hosts was 0.3 cm vs. 0.1 cm on the other host species. Inhibition of root elongation by isolates was 30% on original hosts and 15% on other host species. Two isolates of *F. roseum* caused rot and root inhibition on all species, but neither symptom was as severe on the other hosts as on the original host. The general evaluation can be made that all host species were more severely attacked by their own isolates, as evidenced by higher infection frequencies, longer rots, and greater inhibition of root growth. Some isolates were more virulent on hosts other than the original, but never was the virulence of such isolates very high. Root growth frequently was stimulated by isolates that were nonpathogenic or only slightly so. White clover and birdsfoot trefoil were susceptible to the greatest number of isolates and crownvetch and orchardgrass to the fewest number of isolates. Even though all test fungi originally were isolated from diseased plant tissue, the majority displayed little or no pathogenicity.

Stresses imposed on red clover reduced elongation of roots as follows: Stemphylium leafspot 50%; darkness 86%; and defoliation 66%. There was no difference between the frequency or severity of rot in roots of stressed or normal plants inoculated with isolates 927 and 1055 of *F. roseum*.

The severity of rot caused by the test set of *Fusarium* isolates in severed taproots of greenhouse-grown plants varied with the isolate and the host species (Table 4). Discoloration in noninoculated roots was limited to the cut surface and was given a 1-mm discoloration value. Although isolate 766 caused no root rot in slant-board inoculations, severed taproots inoculated with this fungus had slightly more discoloration than did control roots, but the rot never extended up the taproot more than 5 mm. None of the plants inoculated with 766 exhibited aboveground symptoms. The other three isolates caused root rot in alfalfa and red clover. The isolates were nearly equal in virulence on alfalfa, but there was a greater range in rot severity caused by these isolates on red clover. More severe rot occurred in red clover than in alfalfa, and plants severely rotted by 927 and 959 were stunted and wilted and some died. Isolate 1055 did not kill any plants in our investigations.

TABLE 2. Length of rot[a] and elongation of roots of Arc alfalfa 4 days after inoculation at two sites with the test set of isolates of *Fusarium roseum*

Cultivar and isolate no.	Tip inoculation[b]		2-cm inoculation[c]	
	Rot (cm)	Root (cm)	Rot (cm)	Root (cm)
None (control)	0.0 A[d]	6.0 A	0.0 A	5.7 A
Avenaceum 766	0.0 A	5.2 A	0.0 A	6.9 A
Acuminatum 927	1.2 B	0.0 B	0.0 A	5.8 A
Acuminatum 1055	1.0 B	0.6 B	0.0 A	6.9 A
Acuminatum 959	1.1 B	0.5 B	0.4 B	6.4 A

[a]Length of rot is the length of root rotted after exposure for 4 days to fungal mycelium cultured on a 1.0 × 1.5-cm rectangle of polyester cloth lying on V-8 agar. The roots were attached to growing plants and maintained between layers of polyester cloth kept saturated with nutrient solution.

[b]Inoculum placed on tip of root.

[c]Inoculum placed on root 2 cm above tip.

[d]Values followed by different letters are significantly different at $P = 0.05$ using Duncan's new multiple range test.

DISCUSSION

Our results generally support the concept that most *Fusarium* spp. associated with crown and root rots of forage legumes lack the ability to initiate root rot on their own. Of the 51 isolates tested for pathogenicity on red

clover and alfalfa root tips, only seven had the capability to cause symptoms individually. We feel this is important, because it may change the outlook on feasibility of selecting plants for resistance to root rot. Because so many different species and strains of *Fusarium* have been associated with the root and crown rot complex, the usefulness of selecting for specific resistance was seriously questioned (9). If only a small number of *Fusarium* actually cause the rot, then the likelihood of selecting for useful specific resistance is increased.

Individual roots inoculated with a standardized and pathogenically characterized set of *Fusarium* isolates provided a technique with which to evaluate *Fusarium*-root interactions. The pathogenicity of the test isolates has remained stable and has given consistent results throughout hundreds of inoculations. Although the responses of red clover and alfalfa to inoculation with the test set were quite similar, the general statement can be made that less root rot occurred on alfalfa than on red clover. The susceptibility of roots of orchardgrass was interesting, because Fusarium root rot is not considered to be a problem on orchardgrass in the field. It is possible that orchardgrass roots are killed by the same fusaria that attack forage legume species, but that orchardgrass regenerates roots at a rate sufficient to compensate for those that are lost, or that in nature roots of orchardgrass do not become infected. Also, because grasses do not depend on a single taproot for winter survival, root loss caused by *Fusarium* may never become as critical as it does for legumes. The overall responses of forage species to inoculation with the *Fusarium* test set agree with the performance of these forages in the field.

The inability of some isolates to penetrate and cause rot in intact roots at sites other than at the tips merits discussion because of the question of the ability of the fusaria to penetrate directly and on the importance of root wounding. Incidence of root rot caused by isolate 959 of *F. roseum* 'Acuminatum', which penetrates roots regardless of site, would not be affected greatly by root wounding. However, with an isolate such as 927, also *F. roseum* 'Acuminatum', that has difficulty penetrating roots except at the tip, the wounding of roots might indeed increase the frequency of penetration. It is possible to arrive at completely opposite conclusions on the

TABLE 3. Pathogenicity of *Fusarium* spp. on original and other hosts inoculated at the root tips

Fusarium spp. and original host	Host species infected	Infection frequency		Length of rot[a]		Root growth inhibition	
		Original host (%)	Other hosts (%)	Original host (cm)	Other hosts (cm)	Original host (%)	Other hosts (%)
F. oxysporum 1)[b]	1,2,8[b]	83	6	0.9	0.1	90	22
F. solani 1)	1,3,8	67	3	0.4	0.1	33	4
F. roseum 1)	4	0	1	0.0	0.1	10	9
F. solani 2)	0	0	0	0.0	0.0	2	+8[c]
F. oxysporum 2)	4,8	0	2	0.0	0.1	9	8
F. oxysporum 2)	2,5,8	50	6	0.1	0.1	48	21
F. roseum 'Acuminatum' 3)	1-6,8	67	17	0.9	0.4	80	46
F. roseum 'Semitectum' 4)	1,2,5,8	0	9	0.0	0.1	11	30
F. roseum 'Gibbosum' 4)	2,6	0	2	0.0	0.1	+20	2
F. moniliforme 4)	4,6,8	17	2	0.2	0.1	2	2
F. roseum 'Equiseti' 5)	0	0	0	0.0	0.0	+15	+4
F. moniforme 'Subglutinans' 5)	1,2,5,8	17	12	0.1	0.2	21	17
F. roseum 'Gibbosum' 5)	3,8	0	6	0.0	0.1	4	15
F. roseum 'Avenaceum' 6)	0	0	0	0.0	0.0	0	+6
F. roseum 6)	1-3,6,8	17	10	0.1	0.2	22	22
F. roseum 'Acuminatum' 6)	1-8	100	32	1.6	1.1	100	65
F. roseum 'Avenaceum' 7)	1-8	100	12	0.3	0.3	96	30
F. roseum 7)	2,4,8	0	7	0.0	0.1	+14	6
F. moniliforme 8)	0	17	0	0.1	0.0	15	+9
F. roseum 8)	2,6,8	83	2	0.8	0.1	75	12
F. roseum 8)	2,7,8	17	5	0.1	0.1	15	10

[a]Length of rot is the length of root rotted after exposure for 4 days to fungal mycelium cultured on a 1.0×1.5-cm rectangle of polyester cloth lying on V-8 agar. The roots were attached to growing plants and maintained between layers of polyester cloth kept saturated with nutrient solution.

[b]Original hosts: 1 = alfalfa, 2 = birdsfoot trefoil, 3 = *Coronilla globosa*, 4 = crownvetch, 5 = orchardgrass, 6 = red clover, 7 = subterranean clover, and 8 = white clover (ladino).

[c]The symbol "+" indicates actual stimulation.

TABLE 4. Development of rot in taproots of alfalfa and red clover inoculated in the greenhouse with the test set of isolates of *Fusarium roseum*

F. roseum cultivar and isolate no.	Vertical rot development[a]	
	Alfalfa (mm)	Red clover (mm)
None (control)	1	1
Avenaceum 766	3	4
Acuminatum 1055	7	8
Acuminatum 959	9	14
Acuminatum 957	9	19

[a]Vertical extent of rot in severed tap roots of potted plants which were inoculated by placement of a 1.0 × 1.5-cm patch of polyester cloth covered with *F. roseum* mycelia (developed while the cloth was on the surface of V-8 agar in a petri dish) in contact with the cut surface.

relationship of wounding and penetration depending upon the isolate used. In the greenhouse test in which wounded roots were used, isolate 927 was the most virulent. Thus, isolate 927 could rot mature roots once inside the tissue, which supports the conclusion from histological observations on roots, that the pathogenicity of this isolate was limited by its inability to penetrate.

The results of our experiments with stress were not expected nor did they agree with what has been published (4, 9, 12, 13, 14). The inhibition of root elongation indicated that our stress levels were severe, but that rot was unaffected. In our experiments plants were not stressed for longer than 2 wk, which was shorter than the stress periods used in other experiments (5, 14) in which stress increased root rot development. Also, root penetration was a factor in our tests, whereas it may not have been in others. We concluded that the duration was as critical as the severity in determining the effect of stress on the development of Fusarium root rot.

We concluded that there was host specificity within the fusarial pathogens of forage legumes, although this specificity was not of a high degree. Also, forage species differed in general susceptibility to Fusarium root rot, with white clover and birdsfoot trefoil being relatively susceptible and crownvetch and orchardgrass being relatively resistant.

With the slant-board method of growing plants, the response of a single plant to several *Fusarium* clones, cultivars, and species at two inoculation sites was determined in 3 to 4 days, and replicate inoculations were made on the same plant. The pathogenicity of individual fungi in inoculations made under slant-board conditions apparently is representative of their performance in severed taproots in the greenhouse and the combination of techniques has potential for use in selection of plants resistant to specific isolates of *Fusarium*.

It should be emphasized that our results were obtained under conditions that were completely different from those that occur in nature and serve to demonstrate what the fungi have the potential to do, rather than what actually happens under field conditions.

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