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INTRODUCTION: Massey (3) in 1927 was the first to report that *Cylindrocladium scoparium* Morgan could parasitize plants. In 1928 Sherbakoff (4) isolated the fungus from a chlorotic red clover plant and from diseased apple roots. Cox (2) proved that C. *scoparium* can cause damping-off, root rot, stem infection, and needle blight on seedlings of several conifers. He also found that *C. scoparium* was highly pathogenic on several species of Leguminosae. Bugbee (1) reported alfalfa to be ideal for detecting *C. scoparium* in soil in the greenhouse because the seedlings damped-off and the fungus sporulated on the seedlings. There is, however, essentially no information on the pathogenicity of this fungus on forage legumes. This study was undertaken to determine what effect *C. Scoparium* in soil would have on germination and stand of alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.) and sweetclover (*Melilotus spp.*)

METHODS AND RESULTS: *Isolation from Agricultural Field Soils.* The workers cited above indicate that *C. scoparium* is probably widely disseminated in soils of the U.S., but no systematic effort has been made to determine whether it might be found in soil of agricultural areas. Such information is necessary for estimating its potential importance as a pathogen of agricultural crops.

Soil samples were collected from 72 agricultural fields throughout Minnesota during the spring and summer of 1962. Red clover seed was planted in the soil samples in 6 in. clay pots in the greenhouse set at 85° F. Damped-off seedlings were plated out on acid potato-dextrose agar (APDA). Roots of the seedlings were washed and sections containing lesions were also plated out. Isolates of *C. scoparium* were obtained from 3 different areas of the state using this method. The fields were located near Morris, Mankato, and St. Paul, Minnesota. Thus, the fungus appears to be widely disseminated in agricultural soils, at least in Minnesota.

Growth of Mycelium at Different Temperatures. Temperature is a major environmental factor affecting pathogenicity of fungi and it is possible that *C. scoparium* may not be important as a pathogen of forage crops because of unusually low or high temperature requirements for growth. To determine the influence of temperature on growth, mycelium of the fungus was placed on APDA plates at 5°, 7°, 8°, 12°, 15°, 18°, 19°, 25°, 27°, 30°, 35° C.

An agar plug 6 mm. in diameter cut from a 7-day-old actively growing culture, was used for inoculum in each plate. Colony diameters were measured on 5 plates at each temperature 7 days after the plates were inoculated.

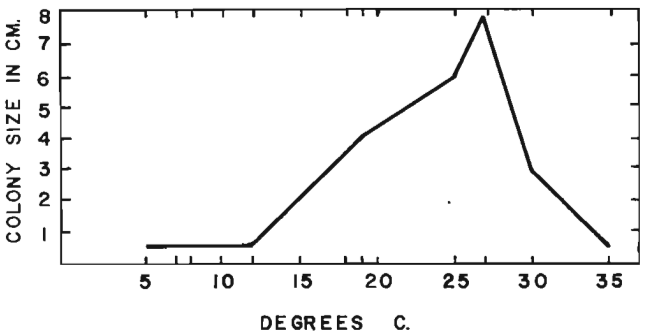

FIGURE 1. Radial growth of *C. scoparium* on APDA at various constant temperatures. (Average of 5 petri plates grown 7 days.)

The fungus grew slightly at 5-12° C. (Figure 1). The optimum temperature for growth was 27° C. At 30° C. growth was reduced appreciably, and 35° C. was the maximum for growth. In 2 other experiments data similar were obtained. Thus, growth occurred at a wide range of temperatures, but the better growth was at somewhat higher temperatures.

Pathogenicity on Forage Legumes. We studied pathogenicity on red clover roots at several different temperatures and also sought information about resistance in varieties of alfalfa, red clover, and sweetclover.

Inoculum was prepared by growing the fungus 15 days on a 1:9 mixture of cornmeal and sterile soil. Soil that was either sterilized or not was then infested with the fungus by mixing it with the inoculum in a 9:1 ratio. Seeds (30/pot), week-old seedlings (10/pot), and month-old plants (2/pot) of red clover varieties Dollard and Wegener were then planted in the infested soil. The pots were arranged at random in the 3 different greenhouses where minimum temperatures were 65°, 75°, and 85° F., respectively. Water was supplied as needed. Disease severity was evaluated 28 days later. Three replicates of each treatment in each temperature were studied. The experiment was repeated, but the use of month-old plants was eliminated. When all of the data were summarized, differences due to the varieties and to the use of sterilized soil appeared to be negligible, and so they were averaged: the data presented in Table 1 indicate the pathogenicity of the fungus at the 3 temperatures.

In all experiments *C. scoparium* reduced stand, vigor, plant height, size of first trifoliate leaf, and root weight when seed or week-old seedlings were planted in infested soil (Table 1). In some cases this reduction was greater

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than 50 per cent. Plant height and vigor of the month-old seedlings was reduced at all temperatures in infested soil and root weight of these plants was reduced at 75° and 85° F., but not at 65° F. Increasing temperatures, in most cases, caused a greater reduction in stand, vigor, plant height, size of first trifoliate leaf, and root weight of the seed and one week-old seedlings. At 85° F. the fungus was favored more than was the plant, since this temperature is closer to the optimum temperature for growth of the fungus. All roots in the infested soil either had necrotic lesions or were completely necrotic, depending on temperature. As the temperature increased the degree of root necrosis increased. Roots in noninfested soil were white and, generally had no necrotic lesions.

Twenty-four varieties of red clover, alfalfa and sweet clover were planted into field soil infested with *C. Scoparium*. One hundred seeds of each variety, replicated four times, were planted in rows in metal flats in the greenhouse set at 85° F. The method of preparing infested field soil was the same as that used in the previous experiment. At 9 days and at 28 days the number of seedlings were counted.

Varying degrees of resistance (percentage of check) were observed among varieties of red clover (Table 2). Ky. Syn. A-1 was most resistant at 9 days and at 28 days; its percentage of check value was 59 and 36%, respectively. The other 7 varieties ranged from 40 to 48% of check at 9 days. There was more variability among these 7 varieties after 28 days. For example, Commercial Medium Red Clover ranked number seven at 9 days and ranked number three at 28 days. On the other hand, Dollard ranked number three at 9 days and ranked number seven at 28 days. The stand at 9 days would represent the reduction due to pre-emergence damping-off in the presence of the fungus, while the stand at 28 days would represent the additional reduction due to post-emergence damping-off. Dollard then had relatively good pre-emergence damping-off resistance and relatively poor resistance to post-emergence damping-off. Commercial Medium Red clover had relatively poor resistance to pre-emergence damping-off but good resistance to post-emergence damping-off.

There was more variability among varieties of alfalfa than red clover (Table 2). W-58 alfalfa from Wyoming was most resistant at 9 days (62%) and at 28 days (52%). Uinta was least resistant at 9 days (30%) and at 28 days (19%). In general, the ranking of varieties was the same at 9 as at 28 days.

Of the sweet clover varieties, Cumino was least resistant. At 9 days the percentage of check value was 18%, while at 28 days it was 11%. The other 3 varieties had about the same degree of resistance.

Pre-emergence damping-off resistance to *C. scoparium* was approximately the same among the 3 legumes; from 43 to 47 per cent. However, alfalfa was most resistant to post-emergence damping-off (Table 2).

DISCUSSIONS AND CONCLUSIONS: Cultural studies showed *C. scoparium* to be a high temperature parasite as far as mycelial growth was concerned. In the greenhouse the fungus was also shown to be more destructive to the forage legumes at higher temperatures. In general, the presence of the fungus reduced stands more than 50 per cent of the check.

Varying degrees of resistance to *C. scoparium* was ob-

TABLE 1. The effect of temperature when seed, week-old seedlings, and month-old plants of red clover were planted in soil infested with *C. scoparium*.[a]

Temp.	Number of plants		Plant vigor ratings[b]		Plant height (cm.)		Size of 1st[c] trifoliate leaf		Root weight (gm.)	
F.	Check	Inoc.	Check	Inoc.	Check	Inoc.	Check	Inoc.	Check	Inoc.
					Seed[d]					
65	17.0	13.5	4.1	2.0	10.0	7.1	2.3	1.4	.13	.07
75	12.5	6.0	4.8	2.9	11.4	4.2	2.9	1.4	.18	.05
85	11.0	2.0	4.4	0.5	11.1	1.1	2.9	0.8	.24	.01
					Week-old[e] *Seedlings*					
65	9.5	4.7	4.5	2.1	12.8	5.9	2.9	1.1	.23	.06
75	9.4	3.4	4.5	1.8	12.5	4.3	3.9	2.7	.27	.04
85	8.5	1.0	4.3	0.6	11.5	1.5	3.7	1.7	.23	.02
					Month-old[f] *Seedlings*					
65	2	2	5.0	4.5	50.0	40.1	—	—	5.51	5.70
75	2	2	4.3	3.0	40.1	28.8	—	—	5.73	3.62
85	2	2	4.5	2.3	34.4	22.4	—	—	3.71	1.43

[a] Two varieties, Dollard and Wegener, which gave similar results were used in the experiment. Both field and sterilized soil, resulting in similar observations, were used in the experiment. The data from these treatments were bulked and treated as replicates.

[b] Based on a 0 to 5 rating; O = no plants, 1 = heavily diseased plants, 5 = healthy plants.

[c] The average size of the first triforliate leaf was determined by measuring the distance between the tips of the two lateral leaflets on each plant.

[d] 30 seeds planted per pot. All figures are averages of 8 replicates.

[e] 10 seedlings transplanted per pot. All figures are averages of 8 replicates.

[f] 2 plants transplanted per pot. All figures are averages of 4 replicates.

TABLE 2. Number of plants of red clover, alfalfa and sweetclover surviving in soil infested with *C. scoparium* after 9 days and after 28 days.

Crop and Variety	% Germination	9 days			28 days		
		Check	Inoc.	% of Check	Check	Inoc.	% of Check
Red clover							
Ky. Syn. A-1	94	79[a]	47	59	81	29	36
Chesapeake	63	54	26	48	54	17	31
Dollard	81	65	29	45	62	13	21
Kenland	90	71	31	44	71	19	27
Lasalle	85	57	24	42	53	15	28
Lakeland	79	65	26	40	65	13	20
Commercial							
Medium Red	95	72	29	40	73	22	30
Pennscott	81	70	28	40	70	19	27
	Ave.	67	30	45	66	18	27
Alfalfa							
W-58	88	66	41	62	65	34	52
Vernal	75	36	21	58	35	15	43
FD-100	80	41	22	54	41	17	41
Culver	91	37	18	49	40	15	38
Cayuga	86	65	30	46	68	23	34
Grimm	84	61	28	46	58	20	34
Narrangansett	75	51	23	45	51	18	35
Minn. Syn. M	81	29	13	45	29	12	41
Ranger	71	35	15	43	33	12	36
Moapa	86	67	25	37	68	18	26
DuPuits	61	14	5	36	14	5	36
Uinta	62	33	10	30	36	7	19
	Ave.	45	21	47	45	16	36
Sweetclover							
Goldtop	20	13	7	54	14	4	29
Evergreen	38	19	10	53	20	6	30
Denta White	71	42	19	45	44	12	27
Cumino	47	17	3	18	18	2	11
	Ave.	23	10	43	24	6	25

[a] Average of 4 replicates.

served among varieties of red clover, alfalfa and sweet clover. Alfalfa was somewhat more resistant than red clover or sweet clover which had approximately the same degree of resistance.

Pre- and post-emergence damage was severe enough that any of the criteria used to measure disease severity: reductions in stand, plant vigor, plant height, size of first trifoliate leaf, or root weight could be used to detect susceptibility in a program of screening for resistance. Plant vigor and stand reduction probably would be most conveniently used.

The disease appeared to be less severe on mature plants than on young seedlings. The results of this study show that *C. scoparium* could be important in reducing stand in forage legumes in nature. The fungus probably is not too important in forage legumes in the cooler portions of the United States, since it is most destructive at 75-85° F. and not so destructive on mature plants. But, in areas where the soil temperature may be 75-85° F. during the seedling stage, the fungus could be important in reducing stand.

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