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SOIL MICROFUNGI INVESTIGATIONS

PAWNEE SITE

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INTRODUCTION

Several recent workers have demonstrated the existence of an abundance of fungal mycelia in both cultivated and undisturbed soils. Jackson (1965) estimated total hyphal length in a New Zealand pasture soil at 125.8 m/g of dry soil in April and 160 m in December. In view of their abundance and their known capacity for decomposition of organic matter, it seems probable that fungi have an important role in elemental and energy cycling within ecosystems. Golley (1960) has estimated that 70% of the net production in a bluegrass old field community is cycled directly to the decomposers, and Macfadyen (1963) in a grazed meadow study estimated that 56% of the energy trapped in photosynthesis was released by decomposers.

An inventory of the dominant microfungi, obtainable by dilution plating, and a quantitative assessment of their relative prominance in the mycoflora seemed to the writers to be a logical first objective in the fungal investigations. Warcup (1951), Brown (1958) and many other workers since the advent of quantitative surveys of soil microfungi have noted the presence of high numbers of species (commonly 100-200 or more dependent on sampling intensity), but in any given stand there appear to be relatively few "prevalent" or widely-occurring forms. Thornton (1956) commented that "...in relatively undisturbed soils a small number of species assume dominance as a result of particular, favourable conditions." Early workers, intent on compiling species lists, felt that there was a constant, characteristic, and cosmopolitan soil microfungal flora (Alexander, 1969). Recent studies, however, have revealed that variations in the existing assemblages of *dominant* species correlate with such measurable community characteristics as vegetation composition, soil moisture parameters, soil pH, and litter calcium content

(Orpurt and Curtis, 1957; Brown, 1958; Thornton, 1956; Warcup, 1951; Christensen, 1969). Species composition in the soil microfungal community, when assessed quantitatively, clearly is influenced by the nature of the cover vegetation and related soil physical and chemical characteristics.

Surveys of the microfungi present in grassland soils have been conducted in Britian, India, Canada, New Zealand, and the United States. The only detailed U. S. grassland studies known to the writers are those of Orpurt and Curtis, 1957 (a quantitative survey of Wisconsin prairie soil microfungi) and England and Rice, 1957 (a survey of the microfungi in virgin prairie and abandoned field soils in Oklahoma). To our knowledge, the Pawnee site study, herein reported, is the first quantitative soil microfungal survey for a shortgrass community in the United States.

Our objectives in this initial survey were (1) to determine the principal species present and their relative frequencies-of-occurrence in one area of the grassland, using the dilution plate technique, and (2) to assess the effect of heavy grazing of the cover vegetation on species composition in the soil microfungal population.

MATERIALS AND METHODS

Surface horizon soil samples (A_1) were collected in August, 1968 at regular intervals along three 250-ft line transects.

The area sampled, a gentle northeast-facing slope in Weld County T10N - R66W - Section 23, is within the Pawnee National Grasslands (IBP intensive study site) of north central Colorado. The principal vascular plant species in the cover vegetation is blue grama (*Bouteloua gracilis*). The soils, which are developing in place in Ogallala sediments, belong to the Ascalon soil series. Soil physical and chemical characteristics are

shown in Table 1. All determinations were made using composite samples collected along two transects in a lightly-grazed portion of Section 23 (Sites A and B) and one in an adjacent heavily-grazed portion (Site C).

For the microfungal population analyses, 10 soil samples were collected at 25-ft intervals along each of the transects. Transects A and B, in lightly-grazed cover, were parallel to one another and about 10 ft apart. Transect C, in adjacent heavily-grazed cover, was laid out continuous with Transect A. The samples were obtained using a tube sampler which was sterilized with 70% alcohol between sample collections. After removal of the vegetation, five to seven soil plugs extending to a depth of about two inches were collected over an area of one square foot. The 30 samples thus obtained were placed in sterile plastic bags, transported in an ice chest, and immediately upon return to the laboratory all were frozen at -10°C. Processing of the samples for microfungal isolation was carried out six weeks later.

A comparison of Pawnee Site populations from fresh soil and from soil frozen for six weeks revealed no significant difference either in number of fungal units or in species composition (Scarborough, 1970).

Sample populations of microfungi were obtained by the dilution plate technique (Orpurt and Curtis, 1957). Soil suspensions in sterile water (1:2500 and 1:5000) were pipetted to petri plates containing acidified Pawnee Site soil extract agar. After incubation for seven days at room temperature, colony counts were made using the plates inoculated with the 1:2500 suspension. Colony numbers on six plates per sample were averaged, and the averages per transect then were used to calculate propagule densities per gram of dry soil. Microfungi were isolated from the plates

inoculated with the 1:5000 suspensions; hyphal-tip transfers to potato-dextrose agar slants were made from 25 sequentially-encountered colonies per sample set. This procedure provided us with 25 cultures per sample, 250 isolates for each transect, and 750 isolates in all.

Selection of lactic acid as the bacterial inhibitor was based upon results obtained in a trial run wherein colony counts (using 20 plates for each treatment) were compared on media containing lactic acid, streptomycin plus rose bengal, and aureomycin plus rose bengal. Colony counts on the acidified medium exceeded counts on the other media by 17-50%.

The tube cultures were incubated at room temperature for 10-14 days, the isolates were sorted on the basis of cultural appearance, and later all questionable separations were checked microscopically. Frequencies (samples of occurrence as a percent of total samples) and densities (isolates as a percent of total isolates) were recorded for all taxonomic entities.

Population comparisons were made using 2w/a+b coefficients of similarity calculations. In that formula, a is the number of species in one population, b is the number in the compared population, and w is the number of species in common. A coefficient of 1.0 (100%) indicates identity.

RESULTS AND DISCUSSION

Grazing Effect

Numbers of colonies per gram dry soil and species composition were very similar in the lightly- and heavily-grazed areas. Thirty-two of the 56 identified forms (Appendix Table 1) were shared species, i.e., present in all three sites, C (heavily-grazed) and A, or C and B. Seven of the 56 taxa were present in the lightly-grazed areas only, and six forms were

present in the heavily-grazed area only. Of the approximately 28 species represented by 10 or more isolates, all but three were present at all three sites.

Calculations of 2w/a+b coefficients using frequency (a and b are sums of frequencies, w is the sum of the lower of the two frequencies for all shared species) are shown in Table 2. The odd vs. even and successive years coefficients (Christensen, 1969) shown at the bottom of that table are indicative of the probable standard error. On the basis of these data, the three populations of prevalent microfungi were judged to be floristically similar.

An examination of microfungal population characteristics at the three sites, however, revealed some interesting differences in the heavily-grazed vs. lightly-grazed areas. Fungal propagule density was slightly higher in the heavily-grazed area, total species present in the 250-membered sample population was lower (54 species vs. 59 and 69), and there were nearly twice as many high-frequency microfungi in the heavily-grazed area in contrast to the lightly-grazed area (Table 3). Disturbance concomitant with heavy grazing apparently effected an increase in the number of prevalent species. England and Rice (1957) reported a similar alteration in the microfungal flora in their Oklahoma study: they recorded four prevalent species (plate densities of 25% or more) from virgin prairie soils and nine prevalent species in soils from an adjacent abandoned field. It may be that disturbance increases the number of primary microhabitats within the soil, thereby promoting a release of subdominants and an increase in number of prevalents.

The principal microfungi obtained from the three transects are listed, in order of declining frequencies, in Table 4 and 5. *Fusarium moniliforme*

was the top-ranking dominant in all three areas and accounted for 91 of the 750 isolates (12.1%). Six other forms -- *Pullularia pullulans*, *Aspergillus fumigatus*, *Cladosporium cladosporioides*, Mycelia Sterilia 4041 (a dark sterile form represented in four of the B samples, frequency 40%), *Trichoderma* variant 4 (site A frequency 40%), and *Ascomycete* 4073 (site A frequency 20%) also were commonly encountered in all transects and their frequencies-of-occurrence do not appear to be significantly affected by grazing of the cover vegetation.

Five of the 12 species shown in Table 4 exhibited frequency variations which may be correlated with grazing. *Hormiscium* sp. 4105 had frequencies of 60% and 30% in the A and B transects, respectively, but was *not* represented in the C transect population. *Penicillium janthinellum*, an abundant form in the C transect, was represented at just one site (frequency 10%) in each of the lightly-grazed area transects; and the last three species in column C (Table 4), all with C frequencies of 50%, also were present in one or both of the lightly-grazed area transects but occurred there at low frequencies (Table 5). *Penicillium janthinellum*, and perhaps the three species just mentioned, appear to be forms which increase in prevalence with grazing.

The Pawnee Site Population and a Comparison with Populations Reported in Other Surveys.

Considering the three transects as a unit, fungal propagule density in the Pawnee Site soil (82,000 - 93,000/g dry soil, Table 3) is less than has been reported for Wisconsin dry alkaline prairie soils (approximately 140,000/g dry soil, Orpurt and Curtis, 1957), but exceeds the range (up to 34,250/g dry soil) reported for western U. S. desert soils (Ranzoni, 1968).

Ninety taxa were represented among the 750 isolates, but only six were obtained from one-half or more of the 30 site samples and only 20 were found in one-fourth or more of the samples (Table 5). Site frequencies, average frequencies, and pooled density figures for 27 species obtained from five or more samples are shown in Table 5. These taxa together accounted for 76.6% of the total isolates.

As can be seen in that table, four Fusaria (*F. moniliforme, F. roseum, F. solani, F. nivale*) ranked among the 20 leading dominants. Species in ***Fusarium*** have been reported to be commonly encountered and abundant in other grassland soils (Scarborough, 1970), but are notably lacking in forest soils, and most are infrequent in desert soils. *Fusarium moniliforme*, present but less frequent than *F. rigidiusculum* and *F. solani* in Oklahoma prairie soils and not recorded from Wisconsin prairie soils, reportedly is a weak seedling parasite on blue grama (Sprague, 1950). *Fusarium roseum, F. solani,* and *F. nivale* are well-known facultative parasites on grasses in general.

Trichoderma variants are known to occur in both forest and grassland soils. In New Zealand, however, Trichodermae ranked among the frequently-encountered forms in debris surrounding old grass roots (Jackson, 1965).

Aspergillus fumigatus was isolated from Oklahoma and New Zealand grassland soils, and was a characteristic species in the dry alkaline Wisconsin prairie soils. This species, in addition, is a common form in desert soils of the western U. S. and Africa.

Pullularia pullulans and *Cladosporium cladosporioides* have been reported for desert soils in New Mexico, Nevada, or both (Durrell and Shields, 1960; Ranzoni, 1968).

Arthrinium arundinis, apparently not common in soil, has been isolated from British dune sands (Brown, 1958). All known isolations of *Peyronellaea* and *Syncephalastrum racemosum*, both of which are distinctive and striking forms morphologically, have been from warm arid soils in the eastern hemisphere and U. S.

No species in *Mucor*, *Absidia*, *Zygorhynchus*, or *Mortierella* were obtained in the present study (Appendix Table 1) although species in all of these genera are known to be common soil inhabitants.

A comparison of the Pawnee Site microfungal population with populations reported in other surveys has revealed that despite the existence of a few shared dominant species and a larger number of shared infrequents, the *combination* of high-frequency species in the Pawnee Site soils is a clearly distinctive assemblage (Table 6). Only four of the 61 species obtained by Warcup (1951) from British grassland soils were present in the Pawnee Site population; and among the 30 top-ranking Oklahoma prairie soil microfungi and 30 top-ranking Wisconsin prairie soil forms, only seven and eight species, respectively, were represented in the Pawnee Site collection.

It is interesting and undoubtedly significant that six of the seven Pawnee Site-Oklahoma survey shared species were summer dominants in the latter study. Similarly, five of the eight species shared with Wisconsin prairie soils exhibited frequency crests, in Wiconsin, in the dry or dry-mesic prairies.

A considerable number of the Pawnee Site species have been isolated from desert soils. There are 16 Pawnee Site species in Ranzoni's Sonoran Desert list, and five of 30 Sonoran Desert prevalent forms were present in the Pawnee Site population (Table 6). The desert-like

Wisconsin sandbar willow soils and the Pawnee soils shared nine prevalent forms (Table 6). Furthermore, all desert and desert-like surveys known to the writers have reported an abundance of dematiaceous and sphaeropsidaceous fungi, and species in these groups also were commonly encountered in the present study. In the Sonoran Desert, Wisconsin sandbar willow, Pawnee grassland, and Oklahoma prairie studies, respectively, Dematiaceae and Sphaeropsidaceae members contributed 36%, 31%, 31%, and 11% of the total species. As Gochenaur and Backus (1967) and Durrell and Shields (1960) have pointed out, the prevalence of dematiaceous and sphaeropsidaceous forms may be the result of a selection for species possessing thick-walled dark-pigmented hyphae, resistant reproductive structures (e.g., pycnidia, chlamydospores), or both. The microfungi which survive in desert surface soils clearly are able to maintain themselves despite rapid and often extreme temperature fluctuations, intense solar radiation, low soil moisture, and low amounts of organic nutrients.

The Pawnee Site (shortgrass community) soil microfungal flora is a distinctive assemblage, embracing a mid- and tallgrass prairie mycoflora element (Fusaria, *Trichoderma*, *Penicillium lilacinum*, *P. restrictum*, *P. funiculosum*, *Aspergillus terreus*, *A. fumigatus*) and a desert mycoflora element (*Pullularia pullulans*, *Cladosporium cladosporioides*, *Aspergillus fumigatus*, dematiaceous and sphaeropsidaceous forms).

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Table 1. Results of soils analyses.

	Site A	Site B	Site C
Soil Texture	sandy loam	sandy loam	sandy loam
Organic matter content (%)*	3.12	2.38	1.56
Water-retaining capacity (%)	11.7	10.6	9.2
pH (paste)	7.0	6.9	7.0
Total soluble salts* E.C. x 10^{-3}	.3	.5	.5
Phosphorus (lbs./acre)*	40.0	28.0	89.0
Moisture content (%)	1.1	1.0	1.0

*Soils analyses by the Soil Science Section, Division of Plant Science, University of Wyoming.

Table 2. Grazing effect: coefficients of similarity.

2w/a+b coefficients using frequency
(all spp. with 1 frequency of 50% or more)

1. Lightly vs. Lightly (A vs. B)	80.8%
2. Lightly vs. Heavily (A vs. C)	71.9%
3. Lightly vs. Heavily (B vs. C)	74.1%

Wisconsin conifer-hardwoods, 6 stands*

Odd vs. even, Coefficient range 81.9 - 92.6% (89.9)

Successive years, Coefficient range 55.1 - 84.7% (72.0)

*See text.

Table 3. Grazing effect: population characteristics.

	Lightly (A)	Lightly (B)	Heavily (C)
Colonies per g dry soil	87,000	82,000	93,000
Species in 250 isolates	69	59	54
Species with frequencies of 50% or more	6	6	10

Table 4. Grazing effect: principal microfungi.
(Species with frequencies of 50% or more)

Lightly-grazed
(A)

Fusarium moniliforme (90%)

Mycelia Sterilia 4041 (70)

Pullularia pullulans (60)

Aspergillus fumigatus (60)

Hormiscium sp. 4105 (60)

Cladosporium cladosporioides (50)

Lightly-grazed
(B)

Fusarium moniliforme (90%)

Trichoderma var. 4 (90)

Pullularia pullulans (70)

Aspergillus fumigatus (60)

Cladosporium cladosporioides (50)

Ascomycete 4073 (50)

Heavily-grazed
(C)

Fusarium moniliforme (80%)

Pullularia pullulans (70)

Cladosporium cladosporioides (70)

Penicillium janthinellum (70)

Aspergillus fumigatus (60)

Mycelia Sterilia 4041 (50)

Ascomycete 4073 (50)

Papulospora sp. 4044 (50)

Pyrenochaeta sp. 4039 (50)

Penicillium sp. 4012 (50)

Table 5. Frequencies and densities (%) for species present in 5 or more site samples and accounting for ten or more isolates.

Culture #	Isolate	Frequency % Site A	Site B	Site C	Avg.	Density %
4089	*Fusarium moniliforme*	90	90	80	86.7	12.1
4070	*Pullularia pullulans*	60	70	70	66.6	4.8
4011_a	*Aspergillus fumigatus*	60	60	60	60.0	7.4
4049	*Cladosporium cladosporioides*	50	50	70	56.7	3.3
4024	*Trichoderma viride* (variant 4)	40	90	30	53.3	4.4
4041	Mycelia Sterilia	70	40	50	53.3	3.3
4073	Unknown Ascomycete	20	50	50	40.0	2.4
4007	*Hormiscium* sp.	40	30	40	36.7	1.8
3092	*Cephalosporium* sp.	20	40	40	33.3	2.0
4104	*Trichoderma viride* (variant 3)	40	20	40	33.3	2.9
4011_b	*Penicillium janthinellum*	10	10	70	30.0	2.6
3096	*Trichoderma viride* (variant 6)	20	40	30	30.0	1.8
4029	*Alternaria tenuis*	30	20	40	30.0	1.6
4072	*Fusarium roseum*	40	30	20	30.0	1.7
4021	*Fusarium solani*	30	30	30	30.0	1.4
4105	*Hormiscium* sp.	60	30	-	30.0	1.4
4044	*Papulospora* sp.	10	30	50	30.0	1.3
4039	*Pyrenochaeta* sp.	30	10	50	30.0	1.3
4069	*Fusarium nivale*	30	30	20	26.7	1.4
4042	*Papulospora* sp.	20	20	40	26.7	1.3
4098	*Trichoderma viride* (variant 5)	10	40	20	23.3	2.2
4068	*Penicillium lilacinum*	20	20	30	23.3	1.4
4012	*Penicillium janthinellum* series	10	-	50	20.0	2.4
4013	*Arthrinium arundinis*	10	30	20	20.0	1.8
4002	*Penicillium funiculosum* strain	20	20	10	16.7	4.0
3099	*Penicillium funiculosum*	10	30	10	16.7	3.0
4078	*Coniothyrium* sp.	40	10	-	16.7	1.6

Table 6. Compositional similarity.

2w/a+b coefficients using presence

(top 30 vs. 23 Pawnee species)

Indian grassland (Dwivedi)	15%
British grasslands (Warcup)	15%
Sonoran desert (Ranzoni)	18%
British acid dunes (Brown)	18%
British alkaline dunes (Brown)	18%
Indian grassland (Mishra)	20%
Oklahoma prairie (England and Rice)	24%
Wisconsin willow-cottonwood sites (Gochenaur and Whittingham)	30%
Wisconsin prairies (Orpurt and Curtis)	32%
Wisconsin sandbar willow sites (Gochenaur and Backus)	34%
Pawnee site	100%

Appendix Table 1. List of identified isolates.[1/]

Culture #	Isolate	Site A	Site B	Site C
4029	*Alternaria tenuis*	X[2/]	X	X
4013	*Arthrinium arundinis*	X	X	X
$401l_a$	*Aspergillus fumigatus*	X	X	X
4086	*Aspergillus terreus*	-	X	-
4004	*Aspergillus* sp.	X	-	-
4008	*Beauveria bassiana*	X	X	-
3091	*Cephalosporium* sp.	X	X	-
3092	*Cephalosporium* sp.	X	X	X
4083	*Cephalosporium* sp.	X	-	-
4084	*Cephalosporium* sp.	X	X	-
4085	*Cephalosporium* sp.	X	-	-
4049	*Cladosporium cladopsprioides*	X	X	X
4078	*Coniothyrium* sp.	X	X	-
4047	*Diplodia* sp.	-	-	X
4071	*Discosia* sp.	-	-	X
4089	*Fusarium moniliforme*	X	X	X
4069	*Fusarium nivale*	X	X	X
4072	*Fusarium roseum*	X	X	X
4090	*Fusarium roseum* variant	-	-	X
4021	*Fusarium solani*	X	X	X
4091	*Fusarium solani* variant	-	-	X
4027	*Helminthosporium anomalum*	X	X	-
4105	*Hormiscium* sp.	X	X	-
4007	*Hormiscium* sp.	X	X	X
4040	*Hormiscium* sp.	-	-	X
4096	*?Idriella lunata*	-	X	-
3095	Mycelia Sterilia	X	-	X
4032	Mycelia Sterilia	X	X	X
4033	Mycelia Sterilia	X	X	-
4041	Mycelia Sterilia	X	X	X

Culture #	Isolate	Site A	Site B	Site C
4026	Mycelia Sterilia	X	X	X
4034	Mycelia Sterilia	X	X	X
4042	*Papulospora* sp.	X	X	X
4044	*Papulospora* sp.	X	X	X
4066	*Penicillium adametzi*	-	X	-
3099	*Penicillium funiculosum*	X	X	X
4002	*Penicillium funiculosum* strain	X	X	X
4012	*Penicillium janthinellum* series	X	-	X
4011_b	*Penicillium janthinellum*	X	X	X
4068	*Penicillium lilacinum*	X	X	X
4075	*Penicillium piceum*	X	-	-
4079	*Penicillium restrictum*	X	-	-
4025	*Peyronellaea* sp.	X	-	X
4070	*Pullularia pullulans*	X	X	X
4050	*Pyrenochaeta* sp.	X	X	X
4031	*Sphaeropsis* sp.	-	X	X
4094	*Stemphylium* sp.	-	X	-
4015	*Syncephalastrum racemosum*	-	-	X
4100	*Trichoderma viride* variant 1	X	X	X
4102	*Trichoderma viride* variant 2	X	-	-
4104	*Trichoderma viride* variant 3	X	X	X
4024	*Trichoderma viride* variant 4	X	X	X
4098	*Trichoderma viride* variant 5	X	X	X
3096	*Trichoderma viride* variant 6	X	X	X
4099	*Trichoderma viride* variant 7	-	X	-
4103	*Trichoderma album* variant 8	X	X	X

1/ Authorities for all binomials in this list are as given by Raper and Thom (1949), Raper and Fennell (1965), and Messiaen (1959) except for *Alternaria tenuis* Nees., *Arthrinium arundinis* (Corda) Fries., *Beauveria bassiana* (Bals.) Vuill., *Cladosporium cladosporioides* Fres., *Helminthosporium anomalum* Gilman & Albott, *Pullularia pullulans* (de Bary & Low) Berkhout, *Trichoderma viride* Pers. ex Fr., and *Trichoderma album* Preuss.

2/ denotes presence.