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Soil Microfungal Relationships Associated With Grassland Reestablishment

Dennis C. Clarke

SOIL MICROFUNGAL RELATIONSHIPS ASSOCIATED WITH GRASSLAND REESTABLISHMENT

BY

DENNIS C. CLARKE

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This thesis is approved as a creditable and independent investigation by a candidate for the degree, Doctor of Philosophy, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

C. J. Mankin
Thesis Advisor

Date

F. C. Westin, Acting Head
Plant Science Department

Date

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INTRODUCTION

The mixed prairie association has a characteristic soil microfungal flora associated with the cover vegetation (Clarke and Christensen, 1980). Rhizosphere organisms are correlated with the cover vegetation (Christensen, 1969) and a change in the cover vegetation should be accompanied by a change in the organisms in the rhizosphere. Some disturbances that may lead to change in the cover vegetation are tillage, grazing, interseeding, mowing and fires. Christensen's (1980) review of species diversity and dominance in fungal communities indicates that in general these disturbances tend to reduce soil microfungal diversity while increasing the frequency of the remaining species. Other studies have shown that the soil microfungi demonstrate a succession that corresponds to the seral stages of the above ground plants as they progress from a pioneer to a climax vegetational unit (England and Rice, 1957; Brown, 1958; Wohlrab, et. al. 1963; Mallik and Rice, 1966; Gochenhour and Whittingham, 1967; Wallace and Dickensen, 1978; and Widden and Parkinson, 1979).

In agricultural crops the microfungi associated with the roots of a developing crop are influenced by plant age, soil type, position in relation to the root (rhizoplane versus rhizosphere) and response to soil fungistasis - low sensitivity being correlated with pioneer colonization of the root and high sensitivity to secondary or non-colonization of the rhizoplane (Peterson, 1958; Das, 1963; Dix, 1967; Young and Kucharek, 1977; and Odunfa and Oso, 1979). Christensen (1980) analyzed key studies and concurs that these factors affect the diversity of species found in a developing community.

In general as succession proceeds the community becomes more diverse and stable. There is disagreement, however, as to the linearity of these relationships. Louck's (1970) studies of general ecological theory indicate a wave pattern in relation to species diversity as succession carries the community toward a climax. Bazzaz (1975) also concluded that succession-diversity relationships are not linear and offered some explanations for the nonlinearity observed. He felt diversity is increased by vertical and horizontal microenvironmental heterogeneity, niche preemptation and sharing of community resources by species of intermediate importance values. Diversity is decreased through the production of allelopathic and other interference substances.

The current study was undertaken to investigate the microfungal successional changes that occur in old wheat field soils reseeded to grass.

DESCRIPTION OF THE STUDY AREA

Location and Study Area History:

The primary study area was located in Jackson County, South Dakota approximately 1 km north and 0.8 km east of Kadoka. The site was used for grain and forage production for approximately forty years. Prior to reseeding grass, the field had been managed using a three year wheat (Triticum aestivum L.), sudangrass (Sorghum vulgare Pers.) and fallow rotation.

The field was seeded to a mixture of western wheatgrass (Agropyron smithii Rydb.) and green needlegrass (Stipa viridula Trin.) in the fall of 1977. The green needle grass did not become well established. Therefore, the resulting stand is primarily a pure stand of western

wheatgrass and weeds.

Stand counts taken during the course of this investigation are shown in Table 1 (page 32). The data indicates a good level of establishment, 6 to 14 seedlings/m^2 being considered indicative of a good stand establishment level (Cook, et. al. 1967; and Bement, et. al. 1965). The data, Table 1, also indicate the grass is reproducing.

Climate and Soils:

The climate of the area is semi-arid continental. Approximately 38.1-40.6 cm of moisture is usually received annually. The yearly mean temperature is approximately 9.44°C with extremes of approximately -30 to 38°C.

The soils of the area are clay, classified in the Pierre Series (Vertic, Haplustoll, with fine, montmorillonite, mesic) and are derived from bedded shale. Table 2 lists the results of a soils analysis conducted by the Soil Testing Laboratory at South Dakota State University.

METHODS

Samples for soil microfungal analysis were collected as outlined in Table 3. The samples were collected at 10 meter intervals along a 100 meter transect. Care was taken so that at each sampling date the transect followed matched that of the previous dates to reduce variability in sampling due to site differences. At each of the ten sites 10 western wheatgrass plants were removed. These plants were composited from each site and held at 4°C prior to analysis.

Plants from each site were shaken to remove loose soil and roots were placed in sterile water blanks, allowed to soak for a period of approximately 30 minutes and agitated for 1 minute to remove the

remaining soil. The soil washed from the roots in this manner was designated as rhizosphere soil. Soil shaken from the roots was designated non-rhizosphere soil.

The roots were chopped and then washed under flowing tap water for 30 minutes to remove any residual soil plus reduce endemic bacterial populations. Roots were trimmed into approximately 0.5 cm pieces and blotted dry. Twenty root pieces for each site were plated on carrot agar to obtain the rhizoplane population estimate.

Serial dilutions were made from the rhizosphere and non-rhizosphere soil samples. The isolation medium used for these samples was soil extract agar (Tresner, et. al., 1954) with Rose Bengal and Streptomycin (Orpurt and Curtis, 1957).

Isolations were completed within 3 to 5 days. Fungi growing from the root pieces were isolated so that approximately 20 isolates per site were obtained. Fungi from the rhizosphere and non-rhizosphere samples were isolated by selecting the first 20 sequential colonies encountered per site.

The isolates were grown on potato-dextrose agar for approximately two weeks prior to sorting into taxonomic groups beased on cultural similarities. Frequency and relative density were recorded for each taxonomic entity where:

$$\text{Frequency} = \frac{\text{No. sites of occurrence}}{\text{Total sites}} \times 100$$

$$\text{Relative Density} = \frac{\text{No. isolates in a taxonomic group}}{\text{Total isolates in the sample}} \times 100$$

Colony counts obtained from diulation plates for the rhizosphere and non-rhizosphere samples were recorded as the mean of triplicate

counts for each sample site and date. An overall mean was calculated for each sample at each date. Colony counts for non-rhizosphere samples were converted to viable propagules per gram of dry soil using the formula:

$$N = \frac{(a)\ (d)\ (100)}{100-X}$$

Where: n = Number of propagules/gram dry soil

a = Number of colonies

d = Dilution factor

x = % moisture in the soil sample

For rhizosphere soil samples the exact dilution factor was not known prior to plating, thus the water used in the initial root washing was collected along with soil washed from roots. The water was evaporated, the dilution factor calculated and the number of viable propagules/gram dry soil determined.

To compare populations within and between sampling dates importance values were calculated for those fungi that attained a frequency of 30% or a relative density of 5% in at least one sample period using the formula:

Importance Value = Relative Frequency + Relative Density

Where: $$\text{Relative Frequency} = \frac{\text{Frequency of a taxon}}{\text{Total frequency of all taxa in a sample}} \times 100$$

$$\text{Relative Density} = \frac{\text{No. isolates in a taxonomic group}}{\text{Total isolates in the sample}} \times 100$$

The maximum importance value any species can attain is 200 in any sampling period - habitat set. Once these values had been derived, they were used to calculate coefficients of community values (Cox, 1976)

utilizing the formula:

$$2w/a + b$$

Where: w = the sum of the lower of the importance values of species in common

a = the total importance value of the species in one list

b = the sum of the importance values in the second list

The coefficients of community were used to construct a matrix that was used in a factor analysis procedure as outlined by Greig-Smith (1964) to determine the basic patterns of similarity of habitats and the occurrence of successional trends. Sample order was arranged to obtain patterns of similarity coefficients within the matrix with the restriction that the group integrity of rhizoplane (1-7), rhizosphere (8-11), and non-rhizoshpere (12-14) samples were retained.

The separation level for coefficients was based on increments of ten. This technique is essentially that of Sorensen as outlined by Greig-Smith (1964).

RESULTS AND DISCUSSION

Propagule Numbers:

Figure 1 shows the number of fungal propagules in the rhizosphere at each sampling date. Variations within samples at each date was normally in ratio of one to three indicating acceptable uniformity of sampling since the magnitude of variation maybe a factor of two to three (Christensen et. al. 1962 and Christensen and Whittingham, 1965).

Rhizoplane propagule numbers varied with sampling date. The number of propagules/gram dry soil decreased between the initial, October, 1977, sampling period and second, May, 1978, sample (not significant at

the 0.05 level) before increasing significantly by the end of the 1978 growing season. Peterson (1958), Das, (1963) and Odunfa and Oso (1979) also found a variation in rhizosphere propagule numbers in relation to plant age. Peterson and Das found the increase to be a steady progression while the data of Odunfa and Oso is similar to that of the present study. These investigators recorded an initial population increase followed by a decrease then an increase as the crop being monitored, cowpea, matured.

The propagule level decreased significantly (Figure 1) at the beginning of the 1979 sampling season. The population level appears to decrease further at both the July and October sampling dates. While a decrease is to be expected as the population evolves toward a climax unit (England and Rice, 1957) and lower populations are found during the summer months in grasslands (Mallik and Rice, 1966 and England and Rice, 1957) the decrease in the Fall, 1979 sample is considered atypical. Clarke and Christensen, (1980) Mallik and Rice (1966) and England and Rice, (1957) report that microfungal populations reach their highest levels in the spring and fall. Possibly the decreased propagule levels in the fall of 1979 are a reflection of the droughty conditions that existed during the 1979 growing season.

Ratios between rhizosphere and non-rhizosphere populations were 1.6:1, 1.25:1 and 1.65:1 for the October 1977, May 1979 and July 1979 propagule counts respectively (Table 4). These ratios are below the ranges of 6:1 and 2:1 R:N values found by Odunfa and Oso (1979) associated with cowpea roots, and the 6:1 ratios that can be computed from Peterson's (1958) data for red clover and the ranges of 9:1 and 33:1 he reports for wheat. The failure to detect greater rhizosphere-

non-rhizosphere population ratios in this study may be due either to failure to separate the two areas definitively or habitat overlap to a greater degree among the dominant fungi.

The propagule numbers in the October 1977 and 1979 rhizosphere samples and those from the May, 1979 non-rhizosphere sample are near the 93,000/g dry soil Christensen and Scarborough (1969) found for the Pawnee Colorado Prairie. The values are much higher than the 55-65,000 levels reported by Clarke and Christensen (1980) for western South Dakota midgrass prairie rhizosphere soils.

Relationship of Population Density to Diversity:

To more fully understand the shifts in propagule numbers it is necessary to examine the microfungal taxa comprising the rhizosphere and non-rhizosphere populations. Data indicates (Table 4) that while the propagule level in the rhizosphere is from 1.2:1 to 1.6:1 that of the non-rhizosphere the number of taxa may not be appreciably different. Earlier (Clarke and Christensen, 1980) it was speculated that microfungal density and diversity were not necessarily positively correlated, i.e. greater numbers do not necessarily translate to more species.

It would appear that the higher propagule levels for the May and July rhizosphere samples are primarily a response to richness of the nutrient sink and not habitat heterogeneity.

The October, 1977, sample is early in the successional sequence. The 11 species advantage in the rhizosphere, 34 versus 23, (Table 4) may indicate habitat difference as the developing root system exerts its effect on the soil nearest the root surface. With time this differential effect is offset to a large degree by the extensive root growth of the cover vegetation.

Rhizoplane populations (Table 4) were lower in species diversity at all sampling periods, as was found by Odunfa and Oso (1979). It would also appear that species diversity is not static, but as was suggested by Loucks (1970) diversity fluctuates in response to environmental perturbations as it changes with successional stages and at the climax.

Comparing diversity of fungi in this study to those reported by other investigators is difficult since soil microfungal researchers rarely use a standard number of isolates either per site or per study within any community. Comparison of diversities from different studies based on varying sample size is inaccurate since diversity in any microfungal community is a function of sample size (Gouchenhaur and Wittingham, 1967; and Clarke and Christensen, 1980). In general the greater the number of samples - isolates - the greater the diversity of species. Therefore, in this study conversion to a common base number of isolates was used to compare the current data to that of other investigators as comparisons based only on studies of exact sample size are restrictive and comparisons based on species dominants are best used for comparisons of habitat specificity of the various taxa.

To convert the data a constant was derived based on species increment data presented by Gouchenhaur and Whittingham (1967) and Clarke and Christensen (1980). Data in these studies indicates that 200 isolates would detect 72% of the total species at the asymptote level and 63.6% at 150 isolates. Thus a conversion coefficient of 66% was selected to equate populations at a 180 isolate level for diversity comparisons. Table 5 shows the species per approximately 180 rhizosphere isolates

from the Colorado, Wisconsin and Buffalo Gap South Dakota grasslands and those from the October 1979 rhizoplane and rhizosphere and May, 1979 rhizoplane, rhizosphere and non-rhizosphere samples for the current study. The data indicates that the drier western prairies have a greater diversity than the eastern drymesic prairies in respect to the number of microfungal species in the rhizosphere. This was also concluded by Christensen (1980). Christensen hypothesized the greater diversity in these more xeric western grasslands may be an indication of a greater number and variety of microhabitats. That the non-rhizosphere and rhizosphere diversity detected in the current study is below that of the Buffalo Gap and Pawnee levels is probably an indication of the lower diversity in cover vegetation resulting in greater homogeneity of microhabitats at the Kadoka study site.

Soil Microfungal Taxa and Population Comparisons:

Tables 6, 7, and 8 list the principle species isolated from the rhizoplane, rhizosphere and non-rhizosphere populations segments respectively. Frequency and relative density of the taxa listed are also shown. Only those species that attained a frequency of 30% or a relative density of 5% at any sampling date for a population segment were designated as principle species.

Table 9 summarizes the number of species/total species from a sample, percent total species and percent total isolates (= total relative density) the taxa represent. The data (Table 9) indicate that as western wheatgrass becomes established the microfungi that can be designated as major forms change. The percent total species and percent total isolates show greater change. With time principle rhizoplane species become a lesser proportion of the total population while in the

rhizosphere the opposite pattern occurs. Although the magnitude of change 81.8% to 61.2% in the rhizoplane versus 47.0 to 57.9 for the rhizosphere differs, the response of the microfungi appears to be habitat related. The percentage of widely distributed, more prevalent fungi, in the non-rhizosphere soil appears to be fairly constant.

Rhizoplane principle forms exceed 90% of the total isolates at all sampling periods. Values at the initial, October, 1977, and final October, 1979, dates are essentially equal. During the course of the study high frequency, high density rhizoplane taxa accounted for a decreasing proportion of the total fungal taxa but a relatively constant proportion of the total isolates. This relationship indicates an increasing dominance of the habitat by fewer species. It would appear the rhizoplane is a relatively specific habitat. In the rhizosphere the opposite trend with respect to dominance appears to occur. The increased number of major forms account for a greater percent of the total species but a decreasing percent of the total isolates. Possibly the rhizosphere, with its richer nutrient sink,can support more species at higher levels both in terms of distribution and density. It would appear there is a greater sharing of community resources available than is evident on the rhizoplane where fewer major taxa utilize a greater share of the available substrate. These findings for the rhizoplane concur with Bazzaz (1975). He found that with succession in higher plant communities species-increase approached log normal as species with intermediate importance values predominated the developing community. Brown (1958) studying microfungal succession in British dune sands also found the number of common species increased during the early stages of succession.

Non-rhizosphere percent total isolates increased (Table 9) at the final July, 1979, sampling date. The percent species occurring as major forms was constant to decreasing slightly. More samples are needed to fully understand the trend in this habitat.

Importance values, Table 10, were calculated for the taxa listed in Tables 6, 7, and 8. This value was chosen to aid in detecting species habitat specificities. Total importance values for the rhizoplane follow a pattern similar to that of relative density. For the rhizosphere after an initial increase, the total importance value for the samples decreased mirroring the trend of major species prevalence. In the non-rhizosphere segment the proportion of the maximum importance value the major taxa comprised increased with time. This increase indicating fewer forms are able to exploit the non-rhizosphere as effectively as the rhizosphere; a trait in common with the rhizoplane populations.

To compare the populations of the three habitats for succession and species habitat specificity the 2w/a + b coefficient of community (Cox, 1976) was calculated for each sample pair both within and between habitats. The values obtained were placed in a matrix of community coefficients. The table was rearranged (see methods) so that patterns of community similarity and difference could be detected (Table 11). Sample separation was based on increments of 10%.

Using the coefficient of community a value of 85% is considered as replicable in a single community and thus is considered to indicate total similarity. None of the samples compared reached the 85% level. High comparison values obtained were 80.5% and 80.3% for comparisons between samples 1 and 2 and 4 and 6, respectively. Comparisons between

samples 4 and 5 and 5 and 6 gave values in the 70% range. All other comparisons yielded values at 69% and below. Rhizoplane populations (Table 11) are most similar to one another and decrease in similarity to each other with time. The least similarity was between samples 1 and 2 compared to sample 7. The degree of similarity between rhizoplane, rhizosphere and non-rhizosphere samples tends to be less than within the rhizoplane. Rhizoplane-rhizoplane comparisons were mostly in the 50-59 and 60-69% range, mean 57.04. Comparisons between rhizoplane and rhizosphere samples were generally in the 30-39 and 40-49% increments, mean 37.06. Comparisons between rhizoplane and non-rhizosphere samples were normally in the same range and had a mean comparison value of 35.10. When higher similarities were noted, in the 50-59% increment, the comparisons were seasonally related.

Rhizosphere-rhizosphere, rhizosphere-non-rhizosphere and non-rhizosphere-non-rhizosphere comparisons were usually in the 50-59 and 40-49% similarity ranges. The most notable exceptions were the 60-69% increment values obtained for comparisons between samples 9 and 13 and 10 and 14. These comparisons of samples from different root-related habitats were taken at the same date. The mean comparison value of rhizosphere-rhizosphere comparisons was 46.98 and that between rhizosphere and non-rhizosphere samples 49.85. The mean value for non-rhizosphere-non-rhizosphere comparisons was 50.57. This clustering of similarity values between and within the rhizosphere and non-rhizosphere possibly indicates a greater degree of niche overlap in these habitats than occurs between the root surface and the soil zones. Thus we would expect more of the same organisms to be common to both the rhizosphere and non-rhizosphere. It may well be that the three habitats evident in

terms of numbers of organisms and numbers of individuals are not habitats that can be distinguished on the same basis. The rhizoplane is distinct both on a qualitative and quantitative basis from the others. The distinction between the rhizosphere and non-rhizosphere is one of quantitative differences at least in relation to the major microfungal inhabitants of these two soil zones.

Samples 9 and 13 are least like any of the other samples. While sample 13 was similar to samples 10 and 11 in the 50-59% range, sample 9 comparison values never exceeded 31.1 except for the 62.1 similarity level to sample 13. Both samples were obtained in May, 1979. Sample 9 was collected from the rhizosphere segment at that date and sample 13 from the non-rhizosphere. The low similarity of these samples to other samples indicates the rhizosphere and non-rhizosphere were probably being dominated by 1 or 2 species at this time with the suppression of other species to lower levels of occurrence. Reviewing Table 10 supports this conclusion. In these sample segments the populations were dominated by Aspergillus flavipes and other taxa, in particular the Fusaria, were depressed to lower levels than normally might be expected.

In relation to individual species the early rhizoplane populations were dominated by Pythium ultimum and Fusarium equiseti. Other high importance value taxa were Fusarium acuminatum, Bipolaris sativum and a Rhizoctonia-like species. With time these species decreased in importance (Table 10) while Fusarium oxysporum, Embellisia chlamydospora and pycnidial forms, most notably Peyrenellea sp. and Pyrenochaeta sp., became increasingly more important. During the course of the study the Fusaria as a group were the predominant rhizoplane taxa.

Pythium ultimum was not isolated from the rhizosphere population as was also the case for non-rhizosphere samples. At the initial sampling period (Samples 1 and 8) the Fusaria dominated this habitat but did not reach a combined importance value as high as for the rhizoplane segment, 58.1 versus 77.5. At the May, 1979, (Sample 9) period the Fusaria had a combined importance value of 20.7. This decrease in the Fusaria is attributed to a combination of seasonal effects and response to other fungal taxa that are known antagonists of the Fusaria.

Fusaria were generally present in the non-rhizosphere soils at levels exceeding those of the rhizosphere but showing an importance value trend similar to that of the rhizosphere. The decreased importance of the Fusaria in the non-rhizosphere soil at the May 1979 (Sample 13) sampling period can be explained by the same factors extended for the rhizosphere at this date. The Fusaria are analyzed further in a later section.

The Aspergilli were most prominent in the soil zones. Members of this genus being isolated at only two of the seven sampling dates from the rhizoplane. Aspergilli were most prominent in the spring in both the rhizosphere and non-rhizosphere soils reaching a total importance value of 65.8 in the former and 47.4 in the latter (Samples 9 and 13). A similar conclusion concerning seasonality with regard to this taxonomic group was reached by Clarke and Christensen (1980) in their study of mixed-grass prairie. The Aspergilli decreased in importance during the growing season (Table 10) having a total importance value of 11.9 in the final (Sample 11) October, 1979, rhizosphere sample. The principle Aspergillus species isolated during the study was A. flavipes. This species is listed by Clarke and Christensen (1980) as being

characteristic in grassland soils.

The Penicillia are also a major group in the soil zones. The taxonomic group increased in importance throughout the study. In the rhizosphere the Penicillia (Table 10) had a total importance value of 14.0 at the initial sampling period (October, 1977, Sample 8) and had increased to a total importance value of 30.3 at the final sampling period (October, 1979, Sample 11). In the non-rhizosphere soils the Penicillia had a combined value of 16.4 (Sample 12) at the initial sampling period and reached a total value of 36.7 by the May, 1979, sampling period (Sample 13). The Penicillia declined during the next two months in the non-rhizosphere and had an importance value of 16.5 at the final sampling date (Sample 14, July, 1979). Principle Penicillia isolated were P. janthanellum, P. lilacinum and P. nigricans in both soil habitats with P. funiculosum being isolated only from the rhizosphere segment.

Pycnidial forms were not isolated from the rhizoplane prior to November, 1978, (Sample 4) they were, however, present in the soils at the initial sampling period (Samples 8 and 12). Except for Phoma sp. (Sample 12) which was a predominant early non-rhizosphere form, these species were of lesser importance in the soil populations. The data indicate that the taxa are normal soil inhabitants but reach their greatest importance on the root. That they were absent from the initial rhizoplane population estimates but for the most part increase in importance with plant age indicates these species are associated with mature roots and possibly serve as primary decomposing agents of the sloughed cortex material (Sprague, 1950 and Domsch and Gams, 1970). Major rhizoplane pycnidial inhabitants were Pyrenochaeta sp., Peyronellea sp.,

and Phoma sp. in order of decreasing importance. The former two species increased in importance on the rhizoplane with time while Phoma sp. decreased. All three species appeared to decrease with time in the soil habitats.

Chrysosporium pannorum, a taxon isolated only from rhizosphere soils during cool seasons, is of interest. Clarke and Christensen (1980) found this species to be prevalent in the fall and to decrease at other seasons in western South Dakota mixed-grass prairie. Like C. pannorum, the Cephalosporia (= Acremonium sensu Gams, 1971) appear to be primarily rhizosphere soil inhabitants but in contrast to C. pannorum reach their maximum importance in the spring (Table 10, Samples 9 and 13), a finding consistent with that of Clarke and Christensen (1980).

From the combined analysis of the microfungal populations as a whole and at the individual species level it is evident that in the successional grassland studied three distinct populations are present. The separation of the population into rhizoplane, rhizosphere and non-rhizosphere segments requires both qualitative and quantitative criteria. The rhizosphere population is the richest in terms of total species, the rhizoplane the poorest. The rhizoplane is dominated by relatively few species while in the rhizosphere, resources are shared by a greater number of inhabitants. The non-rhizosphere population is more diverse than the rhizoplane but the species present, although similar to the rhizosphere, are not capable of exploiting this habitat to the degree that occurs in the rhizosphere resulting in fewer non-rhizosphere taxa dominating the habitat.

Thus it appears that the rhizoplane population exists as a distinct entity both qualitatively and quantitatively. The rhizosphere and non-

rhizosphere populations, while different from the rhizoplane in these factors, can be best separated from one another utilizing quantitative aspects of the populations. Higher levels of species in common were usually found in the rhizosphere.

The remainder of this section will be devoted to analysis of the Fusaria and Embellisia chlamydospora. The former are afforded special attention because of their dominance of the three habitats considered as the cover vegetation becomes established. The latter is singled out not only because of its prevalence but also because this fungus has not been previously reported at high levels.

The Fusaria:

Fusarium species are the major microfungal inhabitants of the root-zone of grasslands (Orpurt and Curtis, 1957; Thornton, 1958 and 1960; Scarborough, 1970; Kreutzer, 1972; Windels and Kommedahl, 1974; Clarke and Christensen, 1980). Predominant species of the genus associated with grasses are F. oxysporum, F. solani and members of the roseum group, most notably F. equiseti.

Several investigators have studied the occurrence of the Fusaria in relation to their presence on the root surface (rhizoplane) versus the rhizosphere and non-rhizosphere soils. In general there is agreement that the Fusaria as a group are most prevalent on the rhizoplane and decrease in numbers with increasing distance from the root (Peterson, 1958; Odunfa and Oso, 1979; and Reyes, 1979). Kreutzer (1972) evaluated F. solani, F. oxysporum and F. roseum found in association with gramineous plants of the short grass prairie. He found that the taxa differed in their response to the three habitats. F. oxysporum followed the projected trend, F. solani was essentially unpatterned and F. roseum

was found in greatest numbers in the non-rhizosphere soils. Thus, while it is possible that the total pattern of Fusarium occurrence is one of increasing prevalence with closer proximity to the root surface, the response at the individual species level differs.

Fusarium populations also change with plant age. Peterson (1958) investigated the Fusaria associated with wheat roots in this respect. He found the Fusaria accounted for approximately 7% of the total fungi isolated at one week after seeding but by 31 days made up 40% of the total isolates. At 90 days the relative incidence had declined to approximately 30%. Reyes and Mitchell (1962) obtained similar data in their studies of the Fusaria in the rhizosphere of host and nonhost plants, Kommedahl, et. al. (1979) also recorded a similar pattern of colonization of the Fusaria associated with corn roots and stalks during the growing season. These investigators also determined that the pattern of colonization is species related. Odunfa (1979) felt these changes in the Fusaria were related to organic compounds exuded from the roots in varying amounts during the growth stages of the plants.

The relative density of the Fusaria on the rhizoplane of the host plants throughout the study period and in the rhizosphere and non-rhizosphere soils is shown in Table 12. Data obtain at the initial sampling date indicates the soils contained a high endemic population of Fusarium. It is also evident that the Fusaria rapidly colonize the seminal root system of the newly seeded grass plants. The relative incidence of the Fusaria in the rhizosphere at one month after seeding is nearly 7% below that on the rhizoplane and approximately 12% below that of the non-rhizosphere at the same date. Although the differences are not significant at the 0.05 level, this pattern is similar at all

sampling dates where data for each of the three root-related habitats is available. Significant differences between the populations exist in the May and July 1979 samples (Table 12). The greatest number of Fusaria are found on the rhizoplane and the least in the rhizosphere. Lower Fusarium incidence in the rhizosphere versus that in the non-rhizosphere is in contrast to previously proposed pattern (Peterson, 1958; Odunfa and Oso, 1979; Reyes, 1979). This decreased incidence may be due to greater total fungal diversity in the rhizosphere compared to that on the rhizoplane and in the non-rhizosphere soils. Data presented earlier have shown that fungal diversity in general is greatest in the rhizosphere.

The large decrease in total Fusaria in the rhizosphere and non-rhizosphere soils in May 1979 (Table 12) was probably the result of seasonality and response to high populations of fungi reported to be antagonistic to the Fusaria. The Fusaria generally predominate in the summer months (Kreutzer, 1972; Reyes, 1979; Watson, 1966; and Thornton, 1960), but the large reduction detected in this study was probably greater than would be expected by seasonal response alone. When the species list for the total population at this sampling period was reviewed high numbers of Aspergillus favipes, a species listed as antagonistic to the Fusaria (Williams and Kaufman, 1962), were noted. High levels of this taxon plus seasonal effects apparently suppressed levels of the Fusaria.

Table 13 shows the frequency and relative density of the Fusarium species isolated during the study. It appears that the Fusaria as a group are widely distributed across all three habitats and are present in greatest numbers on the rhizoplane, however, the individual species react differently within the habitats. It was also evident that there

was a change in the Fusarium population with plant age.

At the initial sampling F. equiseti was the predominant Fusarium species in all three root-related habitats. F. acuminatum was isolated from the rhizoplane and rhizosphere. The species follows F. equiseti in prevalence on the rhizoplane and like F. equiseti decreased in numbers in the rhizosphere. F. oxysporum was present at low levels and increased in frequency and relative density in the rhizosphere and non-rhizosphere soils. F. solani and F. episphaeria were isolated from soils around the roots.

Only rhizoplane data were collected during the 1978 growing season. F. acuminatum decreased to approximately one-half its original relative density (Table 13). F. equiseti decreased in the spring, increased in June and then declined at the end of the season. F. oxysporum increased during the growing season both in frequency and relative density. By June the taxon was present in all sampling sites and had increased five fold in prevalence since May. In the season's final sample this species was the predominant rhizoplane isolate, although the approximately 7% greater isoltion incidence over F. equiseti was not statistically significant. F. solani was isolated for the first time from the rhizoplane in June. F. episphaeria occurred on the rhizoplane only once (Table 13) and thus it appears the species is not normally a part of the root-surface flora as was suggested by Kommedahl, et. al. (1975).

In May 1979, F. equiseti and F. oxysporum were equal on the rhizoplane. F. acuminatum and F. solani were present at approximately their 1978 levels. The Fusaria isolated from the rhizosphere at this sampling period were, with the exception of F. episphaeria, present at levels below the initial sampling period or absent. This again may be

due to a combination of seasonality and fungal species antagonistic to the Fusaria. The same explanation may also be valid for the low non-rhizosphere populations of these species since 35.4% of the total microfungal isolates at this date were Aspergillus flavipes.

In July 1979 the rhizoplane populations of F. equiseti and F. oxysporum increased (Table 13), although the increase of F. equiseti was not significant. The data indicates a possible reversal of predominance on the rhizoplane by these two species, although the species remained equal in terms of distribution. F. oxysporum was the predominant rhizoplane Fusarium species at the October 1979 sampling. F. acuminatum levels were similar to those from the May 1979 sample. Presence of F. solani was similar to that of the October 1977 sample while F. episphaeria was isolated only from non-rhizosphere soil. The rhizosphere and non-rhizosphere F. equiseti and F. oxysporum populations are of special interest at this sampling period. While F. oxysporum regained its wide distribution in the rhizosphere soil levels of F. equiseti were unchanged in terms of frequency and increased to a relative density of 6.7, a level far below that of F. oxysporum. In the non-rhizosphere soils F. equiseti predominated over F. oxysporum and thus it appears the two species are not mutually compatable and may compete for the same niche.

In October 1979 rhizosphere soils F. equiseti had increased while F. oxysporum had declined. F. solani and F. acuminatum were present in both root-related habitats sampled at this date but had decreased both in frequency and relative density. F. episphaeria was isolated from the rhizosphere soil only, reaching a level near that for the May 1979 sample.

Four factors appear to influence presence of the Fusaria. These are successional status of the population being sampled, sample separation, presence of antagonistic fungi and seasonal effect. During the early growth stages of the cover vegetation it appears that F. equiseti and F. acuminatum are the predominant rhizoplane Fusaria. It would also appear that F. oxysporum increases in prominence from the rhizoplane into the non-rhizosphere soils and F. solani and F. episphaeria are primarily limited to soil habitats. On more mature root systems F. oxysporum emerges as the predominant rhizoplane species, as noted by Kreutzer (1972). Although the species decreases in the soil the amount of decrease relative to the rhizoplane is seasonally dependent. The summer rhizosphere levels exceed those of the non-rhizophere while in the spring and fall the trend is reversed. Kreutzer's (1972) interpretation of F. solani as being nonpatterned in its distribution in relation to the root appears to be correct. The interpretation of levels of fungi collectively considered as the F. roseum group is in question. Kreutzer (1972) indicates members of this group predominate in the rhizosphere and non-rhizosphere soils and increase with distance from the root. This is not the case with F. acuminatum which was isolated only from the rhizoplane and rhizosphere in this study with greatest numbers tending to occur in the rhizoplane. F. equiseti becomes more numerous in the soil than on the root with age, however, the exact nature of the population levels at different distances from the root is seasonally related. In the spring and fall populations on the root increase and may exceed rhizosphere levels. The species increases in numbers in the non-rhizosphere soils and may be the predominant Fusarium inhabitant of this zone.

Because of the shifts in Fusarium population noted in the previous discussion it appears that F. acuminatum and F. equiseti are primary colonizers of the root-surface. These species are later replaced by F. oxysporum as the root ages. Kommedahl, et. al. (1975) believe the former species have low competitive saprophytic ability and thus cannot compete effectively with F. oxysporum for infection sites as the roots age. Also the higher summer temperatures seem to favor F. oxysporum activity. Dix (1967) found F. oxysporum to have a low degree of sensitivity to fungistasis thereby leading to its classification as a secondary colonizer of the root surface. Clarke, (1979) has shown F. equiseti to be insensitive to fungistasis. According to the Dix (1967) system the species can be classified as a pioneer, initial, colonizer of the root.

Embellisia chlamydospora:

Embellisia chlamydospora (Hoes, Bruehl and Shaw) Simmons was first described as a new species of Pseudostemphyllium (Hoes, Bruehl and Shaw, 1965) associated with wheat roots and crowns. The taxon was later transferred by Simmons (1971) to the newly erected genus Embellisia. The soil inhabitating fungus is widespread geographically and has been isolated most frequently from members of the Poaceae family or soils associated with these plants (Hoes, et. al. 1965; Simmons, 1971; Christensen, 1979).

Isolation incidence data reported in this section was collected during experiments utilizing three fields. The field designated field one is the study area described in earlier sections of this paper. Field two lies approximately 0.80 km north of field one. The sod was broken in approximately 1954. Since that time the field has been used

primarily for growing winter wheat. Field three adjoins field two and was broken from sod approximately one and one half years prior to the initiation of this study.

Frequency and relative density were calculated for the isolates from field one. Only relative density was calculated for isolations from fields two and three.

Frequency and relative density of E. chlamydospora isolated from the rhizoplane of western wheatgrass and the rhizosphere and non-rhizosphere soils associated with the roots in field one are shown in Table 14.

The October 1977 sample indicated E. chlamydospora is a naturally occurring soil fungus as reported by Simmons (1971) and Christensen (1979). The taxon reached its greatest distribution, frequency, and highest relative density in the rhizosphere at this date. Although the nearly 5 times greater relative density in the rhizosphere was not significant at the 0.5 level, the higher frequency indicates that the initial root colonization zone of E. chlamydospora is the rhizosphere (Table 14). The frequency of occurrence and relative density on the rhizoplane increased to 90 and 16.6 respectively by November 1978 and fluctuated about these values during the 1979 season. E. chlamydospora reached its greatest relative density in May 1979 (Table 14). This may indicate, although the increase was not significant at the 0.05 level, that the taxon reaches its seasonal optimum in the spring. E. chlamydospora was also found to be most prevalent in the spring by Hoes et. al. (1965).

In May 1979 E. chlamydospora was present at a frequency of 20 and a relative density of 1.6 in the rhizosphere soil and 100 and 23.2 on the

rhizoplane. It was not isolated from the non-rhizosphere soil. This decreasing incidence with increasing distance from the root indicates the primary habitat of the fungus is the root and that the fungus is able to maintain only small active populations in the soil. It appears the fungus has a low relative saprophytic competitive ability in the soil.

In fungicide-nematicide experiments conducted in fields two and three in the spring of 1979 (Clarke, unpublished), E. chlamydospora accounted for 28.9 and 21.6% of the total rhizoplane isolates respectively. Approximately four weeks after planting the control plot isolation incidence values for the taxon were 42.8 for field two and 23.1 for field three. The isolation incidence of E. chlamydospora in these experiments were significantly greater than those of samples obtained from field one at a similar plant growth stage (Table 14). The higher isolation incidence in fields two and three may be an indication of higher endemic E. chlamydospora populations in fields two and three; however, this factor was not tested.

A second explanation for the higher isolation levels may be related to differences in prevailing climatic conditions. In the spring of 1978 the soil moisture level was favorable for seedling growth, whereas in 1979 soil moisture levels were low. Possibly E. chlamydospora is better able to occupy available habitat under low moisture conditions.

Response to soil fungistasis was determined for E. chlamydospora utilizing methods similar to those of Lockwood (1977). A concentrated spore suspension of E. chlamydospora was prepared by washing 14-day old cultures grown on PDA into a beaker of cooled 2% water agar. Clean microscope slides were dipped into the agar-spore suspension, removed

and allowed to dry. The slide cultures were placed in 237-ml styrofoam cups; field soil was added so that the cultures were in contact with soil on both slide surfaces.

Cultures were prepared for steamed and nonsteamed soils with three replications for each treatment. Slides were removed at six-hour intervals, rinsed, and stained with Rose Bengal solution. Spore germination was determined microscopically by counting approximately 100 spores per treatment per replication and expressed as percent germination of total spores counted.

The effects of water, soil extract solution, and soil extract solution plus 0.01 g glucose/100 ml soil extract solution were tested using a technique similar to that reported by Yoder and Lockwood (1973). The solutions were added to separate petri dishes until a sterile filter paper liner was saturated. Boiled cellophane squares, approximately 0.5 cm x 0.5 cm, were placed on the surface and seeded with a concentrated E. chlamydospora spore suspension. Each treatment was replicated twice. Sampling followed the method outlined for soil fungistasis trials.

Results of tests conducted to determine the response of E. chlamydospora to soil fungistasis and nutrient amendment are shown in Table 15.

The data indicate spores germinated readily in sterile water, reaching the 50% germination level within 12 hours. This indicates E. chlamydospora spore germination is probably endogenously controlled. The addition of nutrients, soil extract, and soil extract plus glucose increased spore germination at 6 hours to levels equal to or exceeding those observed at 12 hours in sterile water. After 12 hours, spore germination in these solutions was equal or above those in sterile water after 24 hours. Spore germination values were equal at all sampling

periods for steamed and unsteamed soils and indicate E. chlamydospora is not sensitive to soil fungistasis.

The low sensitivity to soil fungistasis and the ability to respond rapidly to a nutrient source (Table 15) allows E. chlamydospora to act as a primary root colonizer according to the system proposed by Dix (1967).

When data from fungistasis experiments and root isolations are examined together the increased incidence of the fungus with root age in field one can be explained by Garrett's (1970) criteria for a successful root invading fungus. The fungus, although not necessarily a good soil competitor, responds rapidly to a favorable substrate, and thus competes well for available sites on the root.

E. chlamydospora appears to be a major component of the microflora of the root zone of western wheatgrass, however, it has not been recorded frequently in the past. Several factors may be involved in the paucity of reports. Its superficial resemblance to the Helminthosporia and Ulocladia have possibly led to misidentifications prior to Ellis' 1976 taxonomic treatment of the dematiacious hyphomycetes. The fungus sporulates poorly under many cultural conditions (Hoes, et. al., 1965) and thus it has probably been designated as a sterile demaciaceous entity. This is felt to have been the case in studies by Mankin (unpublished) and Clarke (unpublished) of rootzone fungi from mixed grass prairie at the Cottonwood, South Dakota, Range Station. A final factor that may be involved is, except for the high incidence of E. chlamydospora in soil between Artemisia sp. and grass clumps in a sagebrush-grassland near Rocksprings, Wyoming (Christensen, 1979), the fungus is not normally present in high numbers in the soil. Many previous studies of soil fungi have assayed only the soil of the

rhizosphere and thus have failed to detect high populations of this taxon. Previous studies have also determined the taxon is weakly parasitic (Hoes, et. al., 1965), however, it may serve to preempt infection sites and reduce the incidence of more virulent forms.

CONCLUSIONS

Data indicate the microfungi in a successional grassland change in both density and diversity. Initially propagule numbers increase then decrease as the cover vegetation matures. Density appears to be influenced by position in relation to the root, the microfungal taxa in the population and seasonality as well as plant age. Greatest microfungal population density occurs in the spring but specific taxa reach their maxima at other seasons, i.e. while the Aspergilli are most prominent in the spring, the Fusaria are present in greatest number in the summer and Chrysosporium pannorum reaches its peak numbers in the fall. That the microfungal population itself affects density is exemplified by the Fusaria. Members of this genus have lowest total relative density in the rhizosphere, the root-related habitat having the greatest microfungal diversity. In general, greatest numbers of microfungi are found in the rhizosphere, their occurrence probably being a response to the rich nutrient sink of this habitat.

Increased diversity is related to the population segment under consideration. The rhizoplane supports the least diverse population, the rhizosphere the most. That the rhizosphere is more diverse than the rhizoplane and non-rhizosphere is probably also a reflection of richness of the nutrient sink. The abundance of nutrients in this habitat allow organisms usually found in greatest numbers on the rhizoplane or in the

non-rhizosphere to exist, although at lower numbers, in the rhizosphere. Habitat overlap is exemplified by Embellisia chlamydospora. Data indicate the taxon is primarily a rhizoplane inhabitat. It exists in the rhizosphere at lower levels and decreases further in the non-rhizosphere soils, the habitat with lowest nutrient availability.

Species differences between the rhizoplane and the soil habitats are fairly distinct (Table 10) both quantitatively and qualitatively. Differences between the rhizosphere and non-rhizosphere populations are less distinct. The principle species in these habitats being essentially the same but differing in prevalence.

The Fusaria as a group and Embellisia chlamydospora were found to be prominent taxa with wide distribution across the three habitats identified.

The Fusaria, in a South Dakota successional grassland, are most prominent on the rhizoplane, decrease in incidence in the rhizosphere probably due to competition from other fungal species and possibly because of antagonistic interactions with other species, and increase in prominence again in the non-rhizosphere soils. F. equiseti and F. acuminatum are primary colonizers of the rhizoplane. These taxa are later replaced by F. oxysporum in predominance. F. acuminatum appears to be restricted to the rhizoplane and rhizosphere habitats in its distribution. F. equiseti inhabits all three root-related habitats but is most predominant in the soil zones as the plants age and alternates seasonally with F. oxysporum in these habitats. F. solani is essentially nonpatterned in its distribution and F. episphaeria is a soil inhabiting taxon.

Embellisia chlamydospora is a natural component of the grassland

microflora and reaches its greatest prevalence on the rhizoplane. The fungus probably possess low competitive saprophytic ability in the soil, but competes effectively when roots are present. Spore germination is endogenously controlled and E. chlamydospora has low sensitivity to soil fungistasis. These properties allow it to become a primary colonizer of the seminal root of potential host plants.

Table 1. Western wheatgrass stand-counts in the study area expressed as plants/m^2.

Sampling period	Plants/m^2
May, 1978	18.4
June, 1978	14.7*
October, 1979	43.4

*Significant decrease at the 0.1 level.

Table 2. Results of soils analysis[a]

Test	Result
Soil texture	Clay
Organic matter (%)	1.2
pH	7.8
Total soluble salts in mmho/cm	0.3
Phosphorus (lbs/A)	14.0
Potassium (lbs/A)	890.0
NO_3-N (lbs/A)	11.0

[a] Soils analysis conducted by Soil Testing Laboratory, South Dakota State University, Brookings, South Dakota.

Table 3. Schedule of sampling.

Date	Rhizoplane	Rhizosphere	Non-rhizosphere
October, 1977	+ [a]	+	+
May, 1978	+	- [b]	-
June, 1978	+	-	-
November, 1978	+	-	-
May, 1979	+	+	+
July, 1979	+	+	+
October, 1979	+	+	-

[a] Indicates sample collected and analyzed.

[b] Indicates sample collected but not analyzed.

Table 4. Number of taxa present in each root habitat and propagules/gram dry soil for rhizosphere and non-rhizosphere soils.

	Rhizoplane	Rhizosphere		Non-rhizosphere	
Sample Date	# Species	Propagules/g dry soil [a]	# Species	Propagules/g dry soil	# Species
October, 1977	11	88,000	34	52,000	23
May, 1978	13	51,000	-	-	-
June, 1978	10	96,000	-	-	-
November, 1978	18	241,000	-	-	-
May, 1979	21	106,000	30	85,000	34
July, 1979	19	97,000	36	59,000	32
October, 1979	21	84,000	39	-	-

[a] To the nearest thousand.

Table 5. Number of species/approximately 180 isolates in different successional and climax prairies using the constant 0.66 to convert the samples to a common isolate level.

Study	Number of Species
Buffalo Gap, South Dakota (Clarke and Christensen, 1980)	41
Colorado (Christensen and Scarborough, 1969)	41
Wisconsin[a] (Orpurt and Curtis, 1957)	
Dry	34.6
Dry-mesic	35.4
Wisconsin[b] (Wicklow, 1973)	
Dry-mesic	30.4
Kadoka, South Dakota	
October, 1979	
Rhizoplane	21
Rhizosphere	38
May, 1979	
Rhizosplane	21
Rhizosphere	30
Non-rhizosphere	34

[a] Average values from continum intervals.

[b] One year post burn control plot.

Table 6. Rhizoplane taxa attaining at least 30% frequency or 5% relative density at one sampling period.

TAXA	SAMPLE DATE													
	Oct., 1977		May, 1978		June, 1978		Nov., 1978		May, 1979		July, 1979		Oct., 1979	
	F	RD	F	RD	F	RD	F	RD	F	RD	F	RD	F	RD
Alternaria alternata	20	4.5	10	1.0	10	0.6	40	2.1	90	12.3	90	9.9	50	3.5
Aspergillus flavipes									40	3.0				
Bipolaris sativum	40	6.7	50	5.9			50	3.6	50	3.5	20	1.1		
Embellisia chlamydospora	10	1.1	40	3.9	50	4.8	90	16.6	100	23.2	80	13.3	100	14.1
Fusarium episphaeria							30	1.6						
Fusarium equiseti	80	24.7	90	15.7	100	41.0	100	22.8	100	14.8	100	24.3	80	12.6
Fusarium acuminatum	50	11.4	50	4.9	40	2.4	80	5.2	60	6.4	70	6.1	40	4.0
Fusarium oxysporum	30	3.4	40	5.9	100	29.5	100	30.1	90	17.7	100	31.5	100	25.3
Fusarium solani					50	3.6	30	1.6	10	0.5	10	0.6	10	0.5
Gliocladium sp.													80	5.0
Periconia sp.									30	1.5				
Peyronellea sp.							30	1.6	50	3.5	20	1.7	20	1.0
Phoma sp.							40	3.1	20	1.0	10	0.6	20	2.0
Pyrenochaeta sp.							40	2.6	50	4.4	40	2.8	80	9.1
Pythium sp. #1			40	8.8										
Pythium sp. #2													90	11.6
Pythium ultimum	80	30.3	100	38.1	80	8.4	50	4.1						
Rhizoctonia-like	60	7.9	70	12.7	60	6.0					30	1.7	20	1.0
Rhizoctonia sp.	20	2.2					10	0.5			30	1.7	20	1.0
Black sterile #1									30	1.5				

Table 7. Rhizosphere taxa attaining at least 30% frequency or 5% relative density at one sampling period.

TAXA	SAMPLE DATE							
	Oct., 1977		May, 1979		July, 1979		Oct., 1979	
	F	RD	F	RD	F	RD	F	RD
Alternaria alternata	70	4.5	70	8.7	50	4.7	40	4.5
Aspergillus flavipes			100	37.6	80	10.1	60	4.5
Aspergillus terreus			70	4.9				
Bipolaris sativum	30	2.0	10	0.5	20	1.3	10	0.6
Cephalosporium sp. (MRS79-10)			40	6.6			20	2.2
Cephalosporium sp. (MRS79-17)			30	2.2			20	1.1
Chrysosporium pannorum	70	8.0					50	5.0
Embellisia chlamydospora	70	5.0	20	1.6	10	0.7	30	1.7
Fusarium episphaeria			40	3.8			50	4.5
Fusarium equiseti	100	14.6	30	1.6	20	6.7	90	21.8
Fusarium acuminatum	50	3.5			40	2.7	30	1.7
Fusarium oxysporum	90	8.5	30	1.6	90	21.5	50	4.5
Fusarium solani	60	5.0			70	6.0	20	1.1
Monodictys sp.							40	2.2
Penicillium funiculosum	40	2.5			10	0.7	30	2.2
Penicillium janthinellum	40	4.5	20	1.1	40	8.7	50	11.7
Penicillium lilacinum			50	7.1	30	3.4	20	1.7
Penicillium nigricans			30	3.8	10	0.7	30	2.2
Periconia sp.	30	5.0						
Peyronellea sp.			60	3.8	30	3.4	20	1.1
Phoma sp.	40	6.0			30	3.4	20	1.7
Rhizoctonia-like	50	3.0			20	2.0	30	1.7
Trichoderma viride	20	1.0	20	1.0	40	2.7	10	1.1
Black sterile #1	80	12.6			10	0.7		
Mucorales	30	1.5	20	1.3			10	0.6

Table 8. Non-rhizosphere taxa attaining at least 30% frequency or 5% relative density at one sampling period.

TAXA	SAMPLE DATE Oct., 1977 F	RD	May, 1979 F	RD	July, 1979 F	RD
Alternaria alternata	30	1.8	50	5.1		
Aspergillus flavipes			90	24.8	90	16.7
Aspergillus flavus			20	9.6	20	1.2
Bipolaris sativum					30	1.9
Cephalosporium sp. (MRS 70-17)			40	2.6	10	0.6
Chrysosporium pannorum	70	10.2				
Fusarium episphaeria	50	8.4	20	1.3	30	1.9
Fusarium equiseti	90	20.5	40	4.5	100	38.9
Fusarium oxysporum	100	14.5	50	5.1	80	6.8
Fusarium solani	30	1.8	20	1.3	40	2.5
Penicillium janthinellum	60	9.0	70	7.0	60	5.6
Penicillium lilacinum			30	5.7	10	0.6
Penicillium nigricans			50	6.4	10	0.6
Peyronellea sp.			60	1.3	30	0.6
Phoma sp.	70	8.4	10	0.6	30	2.5
Stachybotris atra	10	0.6			30	2.5
Trichoderma viride	10	1.2			30	2.5
Black sterile #2					30	1.9

Table 9. Summation of rhizoplane, rhizosphere and non-rhizosphere taxa attaining at least 30% frequency or 5% relative density at one sampling period.

SAMPLE		SAMPLE DATE						
		Oct., 77	May, 78	June, 78	Nov., 78	May, 79	July, 79	Oct., 79
Rhizoplane	No. Species/ Total Species	9/11	9/13	8/10	13/18	13/21	12/19	13/21
	% Total Species	81.8	69.2	80.0	72.2	61.9	63.2	61.2
	% Total Isolates	92.2	96.9	96.3	95.5	93.3	95.3	90.7
Rhizosphere	No. Species/ Total Species	16/34	-	-	-	16/30	17/36	22/38
	% Total Species	47.1	-	-	-	53.3	47.2	57.9
	% Total Isolates	87.2	-	-	-	87.2	79.4	79.3
Non-rhizosphere	No. Species/ Total Species	10/23	-	-	-	13/34	13/32	-
	% Total Species	43.5	-	-	-	38.2	40.6	-
	% Total Isolates	76.4	-	-	-	75.3	87.3	-

Table 10. Fungal species - isolated from rhizoplane, rhizosphere and non-rhizosphere population segments arranged based on importance values.

TAXA	Rhizoplane							Rhizosphere				Non-Rhizosphere		
	(Sample Number)													
	1[a]	2	3	4	5	6	7	8	9	10	11	12	13	14
Pythium ultimum	49.3	57.3	23.5	10.7	-	-	-	-	-	-	-	-	-	-
Pythium sp. (ultimum?)	-	16.5	-	-	-	-	-	-	-	-	-	-	-	-
Pythium sp. (Lobate sporangia)	-	-	-	-	-	-	22.6	-	-	-	-	-	-	-
Rhizoctonia sp.	7.0	-	-	1.8	-	6.3	2.2	-	-	-	-	-	-	-
Fusarium acuminatum	23.3	14.5	9.9	15.7	13.9	16.9	8.9	7.9	-	7.6	4.6	-	-	-
Rhizoctonia-like	22.6	26.2	17.3	-	-	6.3	3.4	7.4	-	4.4	4.6	-	-	-
Bipolaris sativum	16.2	15.5	-	10.2	9.8	4.2	-	4.7	1.9	3.7	1.6	-	-	5.6
Alternaria alternata	9.3	2.9	2.5	7.3	23.3	23.7	9.6	10.7	18.3	10.8	8.4	5.5	11.0	-
Fusarium equiseti	43.7	33.0	59.9	36.0	27.3	39.7	22.4	23.4	5.7	15.2	30.5	31.6	9.2	50.7
Fusarium oxysporum	10.5	13.6	48.4	43.3	29.0	46.9	37.5	16.5	5.7	32.5	9.3	26.8	11.0	16.6
Embellisia chlamydospora	3.5	11.6	11.2	28.4	35.7	25.6	26.3	11.2	4.3	1.9	4.6	1.8	-	4.3
Fusarium solani	-	-	13.0	5.5	1.8	2.1	1.7	10.3	-	14.5	3.0	5.5	3.7	7.4
Periconia sp.	-	-	-	-	5.3	-	-	7.7	-	-	-	-	-	-
Peyronellea sp.	-	-	-	6.7	9.8	7.9	10.8	-	12.0	7.1	3.0	-	8.3	4.3
Phoma Sp.	-	-	-	8.4	3.5	2.1	4.4	9.5	-	7.1	3.6	17.0	1.8	6.2
Pyrenochaeta sp.	-	-	-	7.9	10.7	9.0	18.9	3.8	1.9	1.9	-	3.7	3.7	1.8
Black sterile #1	-	-	-	-	5.3	-	-	19.7	-	1.9	-	1.8	-	1.6
Gleocladium sp.	-	-	-	-	-	-	14.8	2.8	1.9	1.9	1.6	-	-	-
Monodictys sp.	-	-	-	-	-	-	-	-	-	-	6.1	-	-	-
Penicillium funiculosum	-	-	-	-	-	-	-	6.0	-	1.9	5.1	-	-	-
Chrysosporium pannorum	-	-	-	-	-	-	-	14.2	-	-	9.8	18.8	-	-
Cephalosporium sp. MRS 79-10	-	-	-	-	-	-	-	-	12.1	-	4.1	-	-	-
Cephalosporium sp. MRS 79-17	-	-	-	-	-	-	-	-	6.3	-	3.0	-	7.3	1.8
Trichoderma viride	-	-	-	-	-	-	-	2.8	3.7	7.6	2.1	2.4	-	6.2
Aspergillus terreus	-	-	-	-	-	-	-	-	14.5	-	-	-	-	-
Aspergillus flavipes	-	-	-	-	8.0	-	-	-	51.3	19.9	10.3	-	35.4	27.7
Aspergillus flavus	-	-	-	-	-	2.1	-	-	-	2.5	1.6	-	12.0	3.6
Fusarium Episphaeria	-	-	-	5.5	-	-	-	-	9.3	-	9.3	14.5	3.6	5.6
Penicillium janthinellum	-	-	-	-	-	-	-	8.0	3.8	13.6	16.5	16.4	15.2	12.9
Penicillium lilacinum	-	-	-	-	-	-	-	-	13.9	7.1	3.6	-	9.2	1.8
Penicillium nigricans	-	-	-	-	-	-	-	-	7.9	1.9	5.1	-	12.3	1.8
Stacybotrys atra	-	-	-	-	-	-	-	-	-	-	-	1.8	-	6.2
Sterile black (velvety)	-	-	-	-	-	-	-	-	-	-	-	-	-	5.6
Total Importance Value	185.0	191.1	185.7	187.4	183.4	192.8	183.6	166.6	174.5	165.0	151.4	128.8	143.7	171.7

[a] For key to sample numbers see Table 11.

Table 11. Rhizoplane, rhizosphere and non-rhizosphere population comparisons using the 2w/a + b coefficient of similarity.

Code No[a]	1	2	3	4	6	5	7	8	10	11	9	13	12	14
1	-	80.5	59.8	51.4	51.2	40.3	32.7	37.9	30.1	37.2	14.5	17.6	31.5	35.5
2		-	58.9	51.0	44.9	42.2	33.5	39.8	27.7	33.9	11.2	13.8	31.2	31.1
3			-	63.8	44.9	44.3	47.4	45.0	43.9	35.1	10.1	15.9	43.4	44.2
4				-	80.3	73.1	67.3	50.1	50.7	43.1	21.6	28.4	56.2	47.8
6					-	77.1	69.2	49.1	51.1	42.1	24.9	29.0	45.8	42.4
5						-	66.4	53.8	53.0	43.1	31.1	33.5	46.3	42.1
7							-	47.8	50.0	37.4	58.6	27.7	42.4	31.2
8								-	57.8	56.4	52.3	31.0	52.9	45.3
10									-	57.9	62.1	58.6	55.5	62.5
11										-	43.9	52.3	58.2	57.7
9											-	62.1	21.7	40.4
13												-	39.4	53.9
12													-	58.4
14														-

[a] 1, 8 and 12 Oct. 77 RP, RS and NRS, respectively.
2 May, 78 RP
3 June, 78 RP
4 Nov, 78 RP
5, 9 and 13 May, 79 RP, RS and NRS, respectively.
6, 10 and 14 July, 79 RP, RS and NRS, respectively.
7 and 11 Oct., 79 RP and RS, respectively.

Table 12. Relative densities of the Fusaria of the rhizoplanes, rhizosphere, and non-rhizosphere of western wheatgrass at each sampling period.

Date	Rhizoplane	Rhizosphere	Nonrhizosphere
Oct. 77	39.5 (32-47)[b]	31.6 (24-37.5)	43.4 (36-51)
May 78	26.5 (20.5-33)	--[a]	--
June 78	76.5 (68-82.5)	--	--
Nov. 78	62.3 (54.5-69.5)	--	--
May 79	39.4 (32-47)	6.4 (3.5-11.5)	12.2 (7-17)
July 79	62.5 (54.5-69.5)	36.9 (31-45.5)	50.1 (43-53)
Oct. 79	42.4 (35-49.5)	32.5 (26-40)	--

[a] Sample not analyzed.

[b] Confidence intervals = $p \pm z_{.05}\,(\text{normal})\sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$ (Steel and Torrie, 1960)

Table 13. Frequency and relative density of the Fusarium species in the rhizoplane, rhizosphere and non-rhizosphere of western wheatgrass at each sampling period.

Sample	F. acuminatum		F. equiseti		F. oxysporum		F. solani		F. episphaeria	
	F[d]	RD[e]	F	RD	F	RD	F	RD	F	RD
Oct. 1977										
RP[a]	50	11.4 (6.8-16.0)[f]	80	24.7 (18.4-31)	30	3.4 (0.8-6.0)	0	0	0	0
RS[b]	50	3.5 (0.9-6.2)	100	14.6 (9.4-19.8)	90	8.5 (4.4-12.6)	60	5.0 (1.8-8.2)	0	0
NRS[c]	0	0	90	20.5 (14.6-26.4)	100	14.5 (9.4-19.6)	30	1.8 (0-3.7)	50	8.4 (4.3-12.5)
May 1978										
RP	50	4.9 (1.8-8.1)	90	15.7 (10.4-21)	40	5.9 (2.5-9.3)	0	0	0	0
June 1978										
RP	40	2.4 (0.2-4.6)	100	41.0 (33.8-48.2)	100	19.5 (22.8-36.2)	50	3.6 (0.9-6.3)	0	0
Nov. 1978										
RP	80	5.2 (2.0-8.4)	100	22.8 (16.7-28.9)	100	30.1 (23.4-36.8)	30	1.6 (0-3.4)	30	1.6 (0-3.4)
May 1979										
RP	60	6.4 (2.8-10.0)	100	14.8 (9.6-20.0)	90	17.7 (12.1-23.3)	10	0.5 (0-1.5)	0	0
RS	0	0	30	1.6 (0-3.4)	30	1.6 (0-3.4)	0	0	40	3.8 (1.0-6.6)
NRS	0	0	40	4.5 (1.5-7.5)	50	5.1 (1.9-8.3)	20	1.3 (0-3.0)	20	1.3 (0-3.0)
July 1979										
RP	70	6.1 (2.6-9.6)	100	24.3 (18.0-30.6)	100	31.5 (24.7-38.3)	10	0.6 (0-1.7)	0	0
RS	40	2.7 (0.3-5.1)	20	6.7 (3.0-10.4)	90	21.5 (15.5-27.5)	70	6.0 (2.5-9.5)	0	0
NRS	0	0	100	38.9 (31.8-46.0)	80	6.8 (3.1-10.4)	40	2.5 (0.2-4.8)	0	1.9 (0-3.9)
Oct. 1979										
RP	40	4.0 (1.1-6.9)	80	12.6 (7.8-17.4)	100	25.3 (18.9-31.7)	10	0.5 (0-1.5)	0	0
RS	30	1.7 (0-3.6)	90	21.8 (15.8-27.8)	50	4.5 (1.5-7.5)	20	1.1 (0-2.6)	50	4.5 (1.5-7.5)

[a] RP - Rhizoplane; [b] RS - Rhizosphere; [c] NRS - Nonrhizosphere; [d] F - Frequency; [e] RD - Relative Density

[f] Confidence intervals .05 $p \pm z_{.05}$ (Normal) $\sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$ (Steel and Torrie, 1960)

Table 14. Frequency and relative density of E. chlamydospora from the rhizoplane, rhizosphere, and non-rhizosphere of western wheatgrass in field one.

Sample Date	Rhizoplane		Rhizosphere		Nonrhizosphere	
	F[a]	RD[b]	F	RD	F	RD
October 1977	10	1.1 (0-2.6)[d]	70	5.0 (1.8-8.2)	10	0.6 (0-1.7)
May 1978	40	3.9 (1.1-6.7)	-[c]	-	-	-
June 1978	50	4.8 (1.7-7.9)	-	-	-	-
November 1978	90	16.6 (11.2-22.0)	-	-	-	-
May 1979	100	23.3 (17.0-29.4)	20	1.6 (0-3.4)	0	0
July 1979	80	17.3 (11.8-22.8)	10	0.7 (0-1.9)	20	1.9 (0-3.8)
October 1979	100	14.1 (9-19.2)	30	1.7 (0-3.6)	-	-

[a] F = Frequency; [b] RD = Relative density; [c] Not Sampled; [d] Confidence intervals according to Steel and Torrie (1960). $p \pm z\ .05\ (\text{normal}) \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$

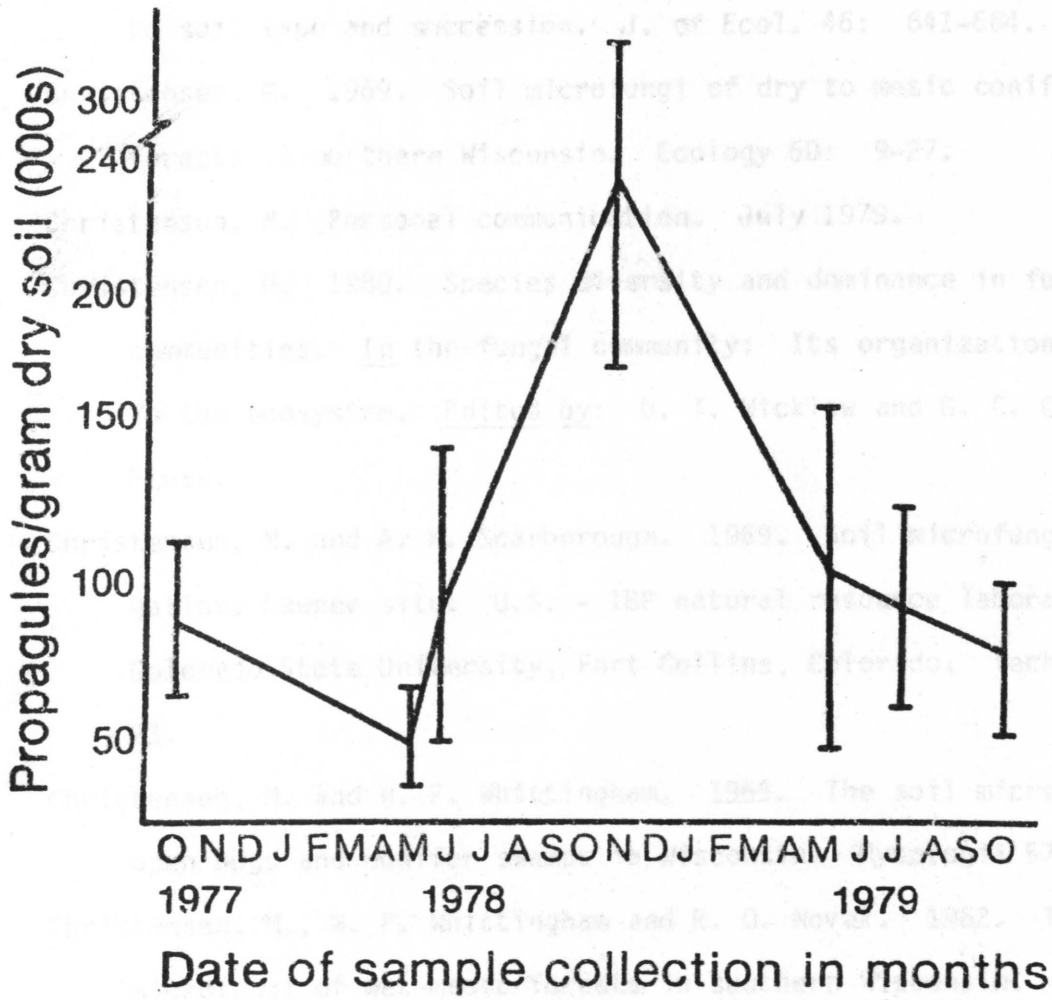
Table 15. Germination percentage of E. chlamydospora spores in relation to soil fungistasis and nutrient solutions.

Time in Hours	Steamed Soil	Unsteamed Soil	Soil Extract	Soil Extract + 0.01g Glucose/ 100ml	Sterile H_2O
6	77 (72.2-81.8)[a]	74.4 (69.5-79.3)	55.8 (48.9-62.7)	73.1 (67.0-79.2)	3.7 (1.1-6.3)
12	100 (98-100)	87.8 (84.1-91.5)	73.1 (67.0-79.2)	89.2 (84.9-93.5)	55.7 (48.8-62.6)
18	95 (92.5-97.5)	97 (96.1-98.9)	63.5 (56.8-70.2)	73.6 (67.5-79.7)	43.4 (36.5-50.3)
24	96.6 (94.5-98.7)	96 (93.8-98.8)	80.5 (75.0-86.0)	87.5 (82.9-92.1)	74.8 (68.8-80.8)

[a] Confidence intervals according to Steel and Torrie, (1960).

$$p \pm z\ .05\ (\text{normal}) \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

Figure 1.
Fungal propagules/gram dry soil in thousands to nearest 1000 from rhizosphere soils.



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