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Factors Influencing the Distribution of Fungi on Plant Roots

Part I—Different Host Species and Fungal Interactions.

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Factors Influencing the Distribution of Fungi on Plant Roots

Part I—Different host species and fungal interactions

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The application of frequencies to the quantitative estimation of fungi on plant roots is discussed. Using this method statistically significant interactions are shown to be present between hosts and root fungi even for those genera which are regarded as being ubiquitous in their habits. Also it is shown that the fungi themselves interact and influence the distribution.

INTRODUCTION

This work has been directed towards the quantitative study of the incidence of fungi on healthy plant roots and their relationships to the rhizosphere. The significant regions of root influence have been differentiated "by using the term rhizosphere to describe the soil region adjacent to plant roots, and the term rhizoplane to denote the plant root surface" as suggested by Clark (1949, pp. 246, 247). The reality of the rhizosphere has been amply demonstrated by many workers, and excellent reviews are available by Clark (*ibid*), Katznelson *et al* (1948).

Quantitative approaches have been used by previous workers to indicate the broad differences between the rhizosphere and soil and between rhizospheres of different species and varieties of plants but there has been little attempt to put the work on a sound statistical foundation. Contois (1953) and Agnihothrudu (1953) are exceptions. Also the fungal relationships of the rhizosphere are not as distinct as those for bacteria, and any more detailed work on that group has been restricted to individual species of fungi of either symbiotic or parasitic interest. Clark (*ibid*, p. 271) states "it remains unsettled whether certain species of fungi are preferentially encouraged by plant roots". The greater difficulty in differentiating the fungal relationships of the rhizosphere is readily understood when we appreciate the fact that fungal mycelium can move a significant distance into an unfavourable medium meanwhile drawing its nutrients from a favourable base. So the boundary lines between ecological microspheres of the soil and root are obscured for this group of microorganisms.

The methods used up to the present to demonstrate quantitative relationships of rhizosphere and rhizoplane fungi fall into five groups as follows—

(1) total colony counts with no attempt to differentiate genera and species, *e.g.*, Katznelson and Richardson (1948),

(2) colony counts for various genera and species, *e.g.*, Timonin (1941) and Agnihothrudu (1953),

(3) colony counts for various physiological groupings, *e.g.*, Atkinson and Robinson (1955),

(4) general statements on the presence or absence of particular genera or groups, *e.g.*, Timonin (1941), Contois (1953),

(5) estimates of the frequency with which certain genera or species occur, in a number of discrete samples, *e.g.*, Katznelson and Richardson (*ibid*), McDonald (1955), Agnihothrudu (*ibid*).

The first four methods have been based on dilution methods and the use of various types of media, modified to make them more or less selective or non-selective. The root material in each case has been fragmented as scrapings from the root surface or by means of a blender, *e.g.*, Stover (1953). The frequency method has tended to be restricted to species associated with root damage, when presence or absence is recorded after making a number of separate isolations from affected tissues. Also it has been considered essential to relate

the findings to a parallel set of figures for uninfluenced soil in a rhizosphere/soil ratio. Though suitable statistical methods for dilution methods have been suggested they are rarely employed owing to difficulties in overcoming variability. Also there is the difficulty as to the significance of fungal counts from dilution methods as discussed by Aberdeen (1955). Of these methods the frequency counts appear to afford the best opportunities for progress providing the underlying fundamentals of frequency methods as used in higher plant ecology are understood, as they offer a sound objective approach which is subject to statistical verification. The following results have been based on these methods and have been applied mainly to rhizoplane communities.

Materials and Methods

Ecological and Sampling Techniques:

The basic procedure was as follows—(i) small root portions of constant surface area were selected at random from roots of higher plants, (ii) each portion was separately washed in several changes of sterile water, (iii) the root fragment was then crushed and/or cut up into small fragments in a sterile petri dish (10 cm.) in a drop of sterile water, (iv) the selected medium at 42° C. was then run into the plates and following solidification incubated at 27° C., (v) the presence or absence of each species of fungus in each plate was recorded and the results used to estimate frequency percentages.

The use of frequency figures (*i.e.*, the percentage of times a species is present in a number of quadrats or samples of fixed size) in soil microbiological work has been discussed previously by Aberdeen (1955) and shown to be a useful method. With the roots some slight modifications are necessary in the mathematical approach to make the method practicable.

As previously it is assumed that Poisson's distribution applies so that

$$a = e^{-m}$$

where a is the proportion of quadrats in which the species is absent and m is the average number of individual units per sample. For root surfaces the quadrat is now a sample area so

$$a = e^{-Ad}$$

where A is the area of the quadrat and d is density of the individual units of point dimensions.

$$\therefore a = e^{-(L2\pi r)d}$$

where L is the length of root fragment and r is the radius, the root fragment being regarded as a small cylinder. If l is the average distance that a fungal colony or growth form extends along the axis of the root and any fungal species is recorded as present in the sample if its colony only so much as touches the sample portion then the result is equivalent to the effective length of the sample being increased by l/2 in both directions and the fungal unit being of point dimensions so that

$$a = e^{-(L + l)2\pi rd} \text{ and}$$

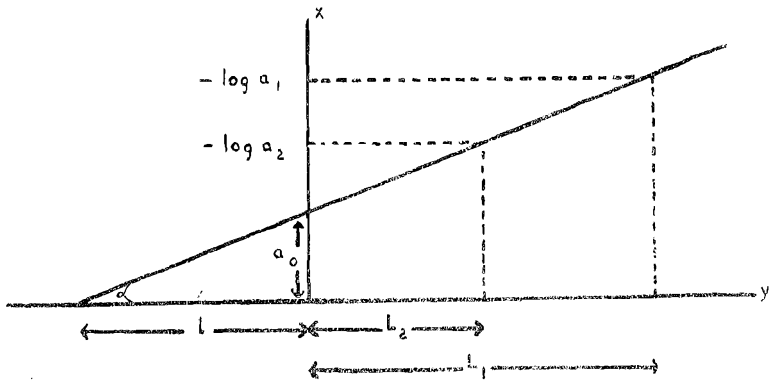
$$-\log a = \log e\,(L + l)2\pi rd.$$

Thus for the same root system, considering roots of equal radius and average colony density of d,

$$-\log a = k\,(L + l),$$

where k is $2\pi rd \log e$. If comparing different sized roots from different species their r must be considered and the comparison be on an area basis. The same relationship, *i.e.*, between $-\log a$ and $(L + l)$ can be used for all root sizes to estimate the proportion of root length occupied.

Fig. 1



Graph illustrating the relationships between root sample length, size of fungal unit and the proportion of samples in which the fungus is absent.
L = root sample length. l = dimension, parallel to the axis of root, of the fungal unit.
a = proportion of samples in which the fungus is absent.

From the equation and the graph

$$2\pi rd \log e = \frac{-\log a}{L + l} = \tan \alpha = \frac{a_0}{l}$$

$$\therefore a_0 = 2\pi rdl \log e$$

$$\therefore \frac{a_0}{2\pi r \log e} = dl = \text{total length of fungus per square cm. of root surface.}$$

The length is the dimension of the fungal unit along the root axis.

The method is illustrated by the following results taken from two plants of *Bidens pilosa* growing as weeds in a garden. Both plants had just commenced to flower, but plant A had been growing in a relatively dry area with intense competition from other weeds and plant B had developed in a relatively moist situation with reduced competition. Twenty root samples each of lengths 0.25 cm. and 1.0 cm. were taken and plated out as above, each cm. sample requiring four petri dishes. Table 1 lists the frequency values with the value for dl for a number of species and Figure 2 shows the corresponding graphs for several selected species. The average dimension of the fungal unit along the root axis and the density of the units can be calculated.

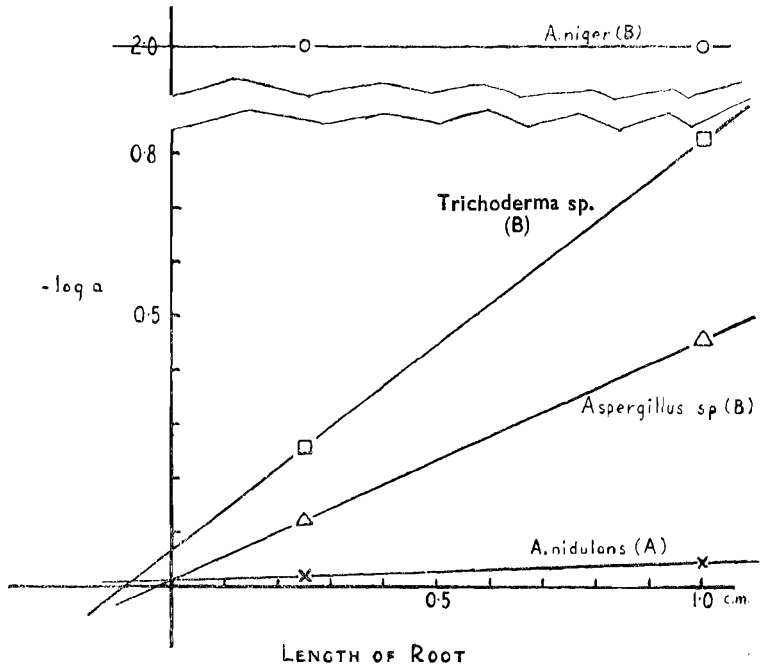
TABLE 1

Frequencies of common fungi on the roots of *Bidens pilosa*

Fungus	Plant A			Plant B		
	Frequency		Length of fungus in cm. per cm. of root (*a*)	Frequency		Length of fungus in cm. per cm. of root (*a*)
	0.25 cm.	1.00 cm.		0.25 cm.	1.00 cm.	
Aspergillus niger	1	8	—ve (*b*)	20	20	3.0 (*c*)
Aspergillus nidulans	1	2	0.02	20	20	3.0 (*c*)
Aspergillus sp.	4	8	0.08	5	13	0.02
Fusarium sp.	2	4	0.04	9	6	0.3 (*d*)
Trichoderma sp.	2	10	—ve (*b*)	9	17	0.1

(*a*) Estimated from graphical results.
(*b*) Negative results.
(*c*) Frequency proportion of 0.99 used for graph.
(*d*) Frequency proportion of 0.375 used for both quadrat sizes.

Fig. 2



Graph showing relationships between - log a and length of root sample

Negative values have occurred and were also present in the soil trials mentioned by Aberdeen (1955). Whether such values are due to an inherent bias in the sampling method or are simply values within an expected range of variation about some small positive value is not known as yet. The conclusion in either case is that the fungus is present as a very small unit.

The comparison of results from a root surface with those from a soil sample has always been a problem in rhizosphere studies. With this method it appears possible that a relative estimate could be made on the basis of percentage of root length occupied against percentage of volume occupied in the soil itself.

For a preliminary investigation one size of quadrat is more convenient as the full method required very large numbers of plates. The above equations and graphs show that the smallest root length possible is to be preferred, the limit of an infinite number of point quadrats giving an exact estimate of the root occupied by a fungous colony. Also it can be seen that a difference in frequency values for a particular size quadrat indicates a difference in the distribution of a species, *i.e.*, of total root occupied and/or size and numbers of units. Equal frequencies, however, can be obtained from either the same or different distributions. Thus in the results below the emphasis can only be put on differences in frequencies.

In the subsequent experiments the root fragments were of the order 4 sq. mm. of root surface. With this size the number of fungal colonies per plate varied from nil to thirty and occasionally more. The high counts were usually due to a preponderance of the colonies from one or a few species. The root systems of the species used in one experiment, tomato and cabbage, are very different macroscopically, the latter being far more dense. When the roots were classified on the system suggested by Cannon (1954) it was found that the final branching in both cases was mainly of the third order, the great overall difference being due to a greater density of the various orders of branches. Roots of the second order were selected from both

plants, the differences in diameter between the two species being balanced by varying the lengths of the samples.

The root system to be examined was carefully removed from the soil and shaken vigorously to get rid of as much soil as possible prior to removal of the samples. Each sample or quadrat was washed three times in successive drops (about 1 cm. diam.) of sterile water in a sterile petri dish by moving it backwards and forwards with a needle for about ten seconds. This treatment is a relatively gentle one and not comparable with the process outlined by Harley and Waid (1955). The major part of this work had been completed prior to this latter article appearing but it was thought advisable to investigate further the effect of a number of these washings on a root fragment. Table 2 shows the average number of colonies and species which were removed in nine successive washings (in groups of three) from ten root fragments of 0.25 cm. each and what was finally left on the fragments. After three such washings there were no fragments of dirt visible to the eye remaining on the roots, so what remained corresponded to the rhizoplane rather than the rhizosphere. As the fragment was transferred from one drop to another some of the liquid went with it so it was possible for spores washed off in the first drop to be carried on for a number of drops. In most cases it reduced considerably the number of colonies of what were presumably the free sporing species.

TABLE 2

Rate of removal of fungi from root surface fragments by ten successive washings

Steps in procedure	Number of species (*a*) present	Number of colonies
Washings 1—3 ..	4	170
Washings 4—6 ..	5	108
Washings 7—9 ..	8	108
On root fragments ..	13	90

(*a*) A species is counted as present in the last step in which it appears.

Cultural Methods:

Throughout the trials the plates were left about 10 days in the incubator, 3-4 days exposed to the light on a table in the room and then all colonies were examined microscopically *in situ*. No attempt was made to name species and only such differences as could be detected macroscopically or microscopically at the time of examination were considered. The purpose of the work was to establish the fact that differences in distribution existed and to dissect out the factors causing the differences. Specific names being a secondary matter, it was sufficient if the various forms could be differentiated consistently.

The medium used throughout these trials was basically that of Czapek's with reduced sugar (0.1%) and additions of "Vegemite" (yeast) extract (0.5%) and Rose Bengal (60 p.p.m.).

Soil quadrats of volume 0.3 cu. mm. were taken by the method described by Aberdeen (1955).

For the order of roots taken from the tomato the root fragments were approximately 2 mm. long. The cabbage roots were relatively longer, to compensate for the smaller diameter. The fragments of one root sample were distributed in each plate.

Results

The Root Community as a Whole:

(1) Under glasshouse conditions.

The experiment was designed to investigate quantitatively the following points:—

(i) differences between fungal communities of soil and of plant root-surface,

(ii) differences between fungal communities on the roots of different species of higher plants,

(iii) the effect of intermingled root systems on the fungal community of each species of higher plant.

TABLE 3

Relative Frequencies of Fungal Species in Soil and on Roots of Tomato and Cabbage

Fungus spp.	Tomato						Cabbage						Both		Soil		Differences	
	alone (5) (*a*)		with cabbage (5)		Mean (10)		alone (5)		with cabbage (5)		Mean (10)		Mean (20)		(5)		Tomato (10) —cabbage (10)	Species (20) —soil (5)
	F.	A.	F.	A.	F.	A.	F.	A.	F.	A.	F.	A.	F.	A.	F.	A.	A.	A.
Cladosporium sp.	15	23.0	19	26.0	17	24.5	15	23.0	14	21.7	14	22.3	16	23.4	5	12.7	2.2	10.7
Gliocladium sp.	9	17.7	13	21.0	11	19.3	25	30.0	31	34.1	28	32.0	19	25.6	11	19.3	−12.7	6.3
Penicillium sp. 1	43	41.3	44	41.5	44	41.4	76	60.5	70	57.1	73	58.8	59	50.1	64	53.3	−17.4	− 3.2
Penicillium sp. 2	14	22.3	13	21.0	14	21.6	21	27.5	19	25.8	20	26.6	17	24.1	46	42.6	− 5.0	−18.5
Penicillium sp. 3	2	9.0	10	18.6	6	13.8	11	19.6	15	22.6	13	21.1	9	17.4	18	25.4	− 7.7	− 8.0
Aspergillus sp. 1	3	10.3	2.5	9.0	3	9.6	11	19.3	1	3.7	4	11.5	3	10.5	40	39.0	− 1.9	−28.5
Aspergillus sp. 2	1	3.7	1	3.7	1	3.7	0	0.0	0	0.0	0	0.0	1	1.8	27	31.6	3.7	−29.8
Aspergillus sp. 3	0	0.0	1	3.7	1	1.8	1	3.7	4	11.0	2	7.3	1	4.5	31	33.8	− 5.5	−29.3
Fusarium spp.	16	23.5	10	18.6	13	21.4	31	33.6	27	31.2	29	32.4	20	26.7	2	9.0	−11.4	17.7
Rhizopus spp.	4	11.6	0	0.0	1	5.8	1	5.3	0	0.0	1	2.6	1	4.2	31	33.7	3.2	−29.5
All fungi ..		16.2		16.3		16.2		22.2		20.7		21.4		16.6		30.0	Differences necessary for significance P = 0.05 10.01 11.3 P = 0.01 13.1 14.7	

F = percentage frequency to the nearest unit. A = average angular equivalent.
(*a*) = number of plots contributing to each average value in the column.

Differences necessary for significance—Average of 5 plots: 5% level 10.1, 1% level 13.1. Average of 10 plots: 5% level 7.1, 1% level 9.3. Average of 20 plots: 5% level 5.0, 1% level 6.6. All means are calculated from angular equivalents and transformed back to frequencies for more direct interpretation.

Treatments were as follows: (i) tomato growing alone, (ii) cabbage growing alone, (iii) cabbage and tomato growing together, (iv) soil with no plants of any species. The four treatments were replicated five times. Ten samples were taken from each root system and the unoccupied soil, making 300 plates to be examined. The soil was relatively poor in nutrients, small dressing of a complete fertiliser being required for the plants to make reasonable growth. This was also reflected in the relatively poor fungal flora obtained. Frequency figures for the ten (10) most frequently occurring fungi considering both soil and root samples, were used. A number of other species were recorded but did not occur in sufficient numbers to warrant quantitative conclusions. No values were taken for the soil present in the containers with the growing plants as it was considered almost impossible to obtain samples free from root influence. Table 3 summarises the results obtained with the analysis of variance in Table 4.

TABLE 4
Analysis of Variance

Source of Variation	D.F.	S.S.	Mean Sq.	F.
Blocks	4	2,593	645	
Hosts	4	6,407	1,602	12.5 xxx
Fungi	9	34,459	3,829	29.8 xxx
Hosts x Fungi—				
Tomato A x	9	685	76.1	—
Tomato B (i)				
Cabbage A x	9	878	97.7	—
Cabbage B (i)				
Tomato x Cabbage ..	9	2,290	255	1.99 x
Plants x soil	9	12,223	1,358	10.6 xxx
Error	196	25,017	128	—
Total	249	84,552	—	—

(i) Tomato A and cabbage A are the plants grown alone and tomato B and cabbage B are those grown together.

x Significant at P = 0.05
xxx ,, at P = 0.001.

Considering the over-all means the soil count is significantly greater than the figure for plants. The absolute figures for soil are not comparable to those for the plants as the soil samples are volume quadrats and the root samples are area quadrats and it is not possible to put them on a common basis, unless ranking methods are used. However, interactions between the two can be studied and will be discussed later. Cabbage significantly exceeds tomato. This difference could be due to four factors—(*a*) differences in total area of the sample quadrats, (*b*) differences in the root hair densities of the surface, which would tend to influence the amount of very fine soil particles retained, (*c*) differences in the shape of the quadrat, the cabbage being relatively much longer, (*d*) differences in the root excretions, *i.e.*, nutritional differences. Care was taken to eliminate (*a*). A number of the samples from the same order of roots as used were examined under the microscope. Root hairs were present to some extent on both. Relative counts were not made but the differences were not noticeable, nor was the difference in adhering particles striking. A subsequent experiment (see Table 5) supported the suggestion of nutritional differences. No further evidence is available on the effect of quadrat shape. Significant differences between the incidence of fungal species either overall or on individual root systems are also apparent.

The analysis indicates no significant differences between higher plants of the same species grown alone or in close proximity to the other species. The interaction between plants and soil is very significant. Three species of *Aspergillus*, one of *Penicillium* and *Rhizopus* clearly favour the soil. With *Fusarium*, *Cladosporium* and *Gliocladium* favouring the root surface. In addition to nutritional differences due to the presence and absence of roots the straight soil would not be subject to the same fluctuation in moisture as for the pots in which plants were growing.

The interaction between higher plants and fungi was also significant, with *Cladosporium* and possibly *Aspergillus sp.* 2 and *Rhizopus sp.* favouring tomato and the remainder, particularly *Gliocladium sp.*, *Penicillium sp.* 1 and *Fusarium spp.* favouring the cabbage.

Cladosporium sp. was selected from the first group being much commoner, and *Gliocladium sp.* from the second group and the growth of both fungi compared on extracts from the root systems of the actual plants analysed for frequencies. The root systems of the ten tomato and cabbage plants had been retained and at the close of frequency estimates were soaked for approximately six hours in 250 cc. of cold water. The resultant liquid was poured off the roots, the soil allowed to settle and the supernatant liquid made up to 300 cc. of agar medium with 0.1% sucrose, sterilisation being effected by autoclaving at 15 lbs. for 10 mins. Table 5 shows the growth after seven days, growth of *Cladosporium sp.* showing no significant differences between the hosts and the *Gliocladium sp.* distinctly favouring the cabbage root extract, the order of difference in terms of areas of colonies being closely comparable to the order of difference shown by the relative frequencies.

TABLE 5

Growth of *Cladosporium sp.* and *Gliocladium sp.* on tomato and cabbage extracts

Fungus	Tomato root extract sq. cm.	Cabbage root extract sq. cm.	Significance
Cladosporium sp.	2.56	2.75	N.S.
Gliocladium sp.	13.6	22.8	$P<0.001$

(2) In the field.

By the same methods a comparison was made of fungal flora on the roots of Rhodes Grass (*Chloris gayana*) and *Tagetes minuta* from two different districts approximately 12 miles apart. In the experiment B the grass mat was several years old with the *Tagetes* a typical annual, growing amongst it. In Experiment A the grass sward was younger, approximately 12 months. In both experiments the pairs of plants in each replication were from 3-12 inches apart, each pair being separated from any other pair by a greater distance than 12 inches. Both species were in flower. Ten replications were used and 15 samples taken from each plant root system, *i.e.*, 300 plates were examined for each experiment.

TABLE 6

Frequencies of fungi on root surfaces of *Tagetes* and *Chloris* (Experiment A)

Fungus	*Tagetes*		*Chloris*		Differences
	Ang. equiv.	Freq. (*a*)	Ang. equiv.	Freq. (*a*)	Ang. equiv. T—C
Cladosporium sp.	16.8	8	12.3	5	+ 4.5
Gliocladium sp.	18.2	10	16.4	8	+ 2.2
W 43	19.8	11	20.3	12	— 0.5
Fusarium spp.	14.8	7	17.0	9	— 2.2
Aspergillus sp. 2	20.1	12	22.5	15	— 2.4
Aspergillus sp. 1	31.9	28	34.5	32	— 2.6
G 59	18.7	10	23.0	15	— 4.3
B 1	13.0	5	22.4	14	— 9.4
Penicillium sp. 1	16.1	8	25.7	19	— 9.5
Penicillium sp. 3	12.1	4	21.7	14	— 9.6
Trichoderma sp.	13.7	6	26.3	20	—12.6
Differences nec. for significance (ang. equiv.) $P = 0.05$			8.8		12.4
$P = 0.01$			11.5		16.2

(*a*) to nearest unit.

Tables 6 and 7 list the results with analyses of variance for the eleven commonest fungi occurring in experiment A. Significant differences are present for fungi, hosts and the interaction between host and fungi. Tables 8 and 9 show the results for the six commonest fungi in experiment B. Differences between fungi are again emphasised, with the overall difference between hosts not significant and the host x fungus interaction just under significance at the level P = 0.05. The main interest is the host x fungus interaction, the two fungal species *Cladosporium* sp. and *Trichoderma* sp. showing the same order of results independent of the different environmental factors for the two trials. The soil factor was clearly different and a second important difference was the large proportion of old roots in the *Chloris* sward for experiment B. This was probably one of the main factors in obscuring the significance of the interactions in this trial. In both experiments the species with frequencies greater than five per cent. on one host were included in the statistical analysis.

TABLE 7

Analysis of Variance

Source of Variation	D.F.	S.S.	Mean Sqq.	F.
Blocks	9	2,099	233	
Fungi	10	20,563	2,056	10.3 xxx
Hosts	1	4,227	4,227	22.2 xxx
Hosts x Fungi	10	5,814	581.4	2.92 xx
Error	189	37,583	198.8	
Total	219	70,286		

xx P < 0.01. xxx P < 0.001.

TABLE 8

Frequencies of fungi on root surfaces of *Tagetes* and *Chloris* (Experiment B)

Fungus	*Tagetes*		*Chloris*		Differences Ang. equiv. T—C
	Ang. equiv.	Freq. (*a*)	Ang. equiv.	Freq. (*a*)	
W 8	13.9	6.0	9.7	3.0	+ 4.2
Cladosporium sp.	15.4	7.0	13.2	5.0	+ 2.2
Penicillium sp. 1	19.0	11.0	17.8	9.0	+ 1.2
B 3	10.3	3.0	15.6	7.0	— 5.3
Trichoderma s.p.	15.2	7.0	21.5	13.0	— 6.3
Differences nec. for significance (ang. equiv.) P = 0.05			7.9		11.2

(*a*) to nearest unit.

TABLE 9

Analysis of Variance

Source of Variation	D.F.	S.S.	Mean Sqq.	F.
Blocks	9	3,247.2	360.8	
Fungi	4	2,736.8	684.8	4.19
Hosts	1	36.0	36.0	—
Hosts x Fungi	4	1,583.5	395.9	2.42
Error	81	13,244.1	163.4	
Total	99	20,847.6		

P = 0.05 for F = 2.48.

Analysis of interactions between fungi.

The overall significance of the interaction between hosts and any particular fungus is apparent from the above trials. Interactions between fungi, however, cannot be discerned. To investigate this aspect the 22 commonest species of the fungi isolated in experiment A were investigated for significant associations. This experiment was selected because of the relatively large number of records for each fungous species, *i.e.*, 300, and because it showed greater significant differences than the parallel experiment B. χ^2 tests were used to calculate the significance of the association and the index of association was calculated according to the formulae of Cole (1949) as it was considered that his arguments in favour of that index were applicable to this experiment. Table 10 lists the commonest species as given in Table 6, along with one other that showed significant interactions. A number of other significant differences were also noted in the remainder of the 22 species investigated.

TABLE 10

The significance and the index of association between fungal species on the roots of *Chloris gayana* and *Tagetes minuta*

Host	Host	Fungal species											
	Chloris gayana / *Tagetes minuta*	*Trichoderma sp.*	*Aspergillus sp. 1*	*B 1*	*Cladosporium sp.*	*Penicillium sp. 3*	*Penicillium sp. 1*	*Fusarium spp.*	*B 2*	*G 59*	*Aspergillus sp. 2*	*W 43*	*Gliocladium sp.*
Fungal species	*Trichoderma sp.*		*	x −.33	xx −.33			x +.29	x −.21	x +.09	*		
	Aspergillus sp. 1			* +.75			x +.34	x −.29	x +.48				
	B 1		x +.81								*		*
	Cladosporium sp.							*					
	Penicillium sp. 3												
	Penicillium sp. 1		xx +.63			x +.27			x +.21				
	Fusarium spp.									*			
	B 2		xx +.86			*				x +.18			*
	G 59	x +.45	xx +.66	*									
	Aspergillus sp. 2		xx +.51				*		xx +.44	*			
	W 43				*								
	Gliocladium sp.			x +.21		x +.18							

* = 0.10 >P>0.05
x = 0.05 >P>0.01
xx = 0.01 >P>0.001
xxx = 0.001>P

TABLE 11

Significance of interaction between fungal species in the presence of two different hosts

Interaction	χ^2 values			
	Total	Fungus x fungus	Fungus x host	Residue (by subtraction)
Trichoderma sp. x .. *Aspergillus sp.* 1 x hosts	53.2 xxx	0.2	T x H 49.2 xxx A x H 3.1	0.7
Trichoderma sp. x *Cladosporium sp.* x hosts	65.0 xxx	8.7 xx	T x H 49.2 xxx C x H 4.4 x	3.3
Cladosporium sp. x *Aspergillus sp.* 1 x hosts	8.0 x	0.1	C x H 4.4 x A x H 3.1	0.4
Aspergillus sp. 1 x *Penicillium sp.* 1 x hosts	41.7 xxx	14.2 xxx	A x H 3.1 P x H 23.8 xxx	0.6
Penicillium sp. 1 x *Trichoderma sp.* x hosts	76.9 xxx	4.7 x	P x H 23.8 xxx T x H 49.0 xxx	0.4
B 1 x *Asperigillus sp.* 1 x hosts	11.4 x	8.3 xx	A x H 3.1 B1 x H 0.2	0.0
B 1 x *Trichoderma sp.* x hosts	54.7 xxx	2.7	T x H 49.2 xxx B1 x H 0.2	2.6
B 1 x *Penicillium sp.* 1 x hosts	25.6 xxx	0.7	B1 x H 0.2 P x H 23.8 xxx	0.9

x 0.05 >P>0.01
xx 0.01 >P>0.001
xxx 0.001>P

The striking fact shown in Table 10 is the change in significance and/or the index of the association between two fungal species as the results are analysed for the separate root systems. To enable the fungal interaction to be separated from host interaction the results for (1) occurrences together, (2) separate occurrences, and (3) neither species occurring for each pair of fungi on each hosts were set out as 2 x 2 x 2 tables and analysed for significance by χ^2 test which was afterwards partitioned to separate the fungus x fungus interaction from the fungus x host interactions.

Table 11 shows the results of such an analysis for a number of species from this particular experiment. The striking interaction between *Trichoderma* sp. and the two hosts is again apparent. The fungal interactions are now clearly indicated. The case of *Trichoderma* sp. has further interest in that as well as showing a greater preference for *Chloris gayana*, the significant negative association with several other fungal species on the roots of that host disappears on the roots of *Tagetes minuta*. *Trichoderma* sp. and *Penicillium* sp. 1 have a similar host interaction but are sharply differentiated by their interactions with *Aspergillus* sp. 1.

Several results were taken from field experiment B and from the glasshouse trial and analysed in a similar way. The total χ^2 values for the overall interactions in experiment B, *i.e.*, fungus x fungus x host, were just under significance at the level P = 0.05, as had already been shown to be the case by the earlier analysis of variance. If, however, the χ^2 is partitioned the same order of results is obtained for field experiment B, the *Trichoderma* sp. x *Cladosporium*

sp. x hosts interaction again emphasised the *Trichoderma* x host interaction. With the glasshouse experiment the *Gliocladium* x *Cladosporium* x host interaction centred mainly on the *Gliocladium* x host interaction.

Discussion

The overall influence of the host plant on fungal population of the rhizoplane has been demonstrated before, *e.g.*, Aginhothrudu (*ibid*), but there has been no attempt to analyse these interactions in further detail as far as I am aware.

The above results show clearly that the distribution of fungi on the roots of higher plants is influenced by at least two factors: (1) the host plant and (2) the associated fungi. The extent to which each of these factors influences the final distribution varies. In the case of the *Penicillium* sp. 1 x *Trichoderma* sp. x host interaction, the most significant contributions are the interactions of the individual fungal species with the hosts. For the *Penicillium* sp. 1 x *Aspergillus* sp. 1 x host interaction it is the *Penicillium* sp. 1 interaction with the hosts combined with a significant positive association of the two fungal species. The B1 x *Aspergillus* sp. 1 x host result is due almost entirely to the high positive association of the two fungal species.

Discussion of such results could be extended considerably as a number of fungal species other than those listed showed significant interactions on one host or the other. Also if the investigator was interested in one particular species then more complicated interactions involving more than one other fungal species could be investigated.

It should also be noted that for the species listed in Table 10 there are 132 possible associations and that on random variation alone there is an expectation of between six and seven results exceeding values which indicate a probability of $P = 0.05$. The actual total exceeding this standard is 20. For $P = 0.001$ the expected is between one and two and the actual is six.

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