Ecology and Epidemiology

Colonization of Field-Grown Cotton Roots by Pathogenic and Saprophytic Soilborne Fungi

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I thank F. N. Martin, R. S. Kinsbursky, and S. T. Baba for technical assistance.
The assistance provided by the laboratories of J. G. Hancock, A. R. Weinhold, and R. G. Pratt in the identification and soil assays of the *Pythium* species and *Rhizoctonia* species is gratefully acknowledged.
Accepted for publication 8 December 1987 (submitted for electronic processing).

ABSTRACT

Huisman, O. C. 1988. Colonization of field-grown cotton roots by pathogenic and saprophytic soilborne fungi. Phytopathology 78:716-722.

Colonization of roots of field-grown cotton plants by several fungi (*Verticillium dahliae*, *V. tricorpus*, *Gliocladium* sp., *Trichoderma* spp., *Rhizoctonia* spp., *Pythium ultimum*, *P. aphanidermatum*, and an unidentified *Pythium* sp.) was examined by plating washed roots on a growth restrictive medium. Most colonies were localized on the roots and apparently were associated with the rhizoplane or the outer cortex. With the exception of *Pythium* spp., which colonized roots only during periods of high soil moisture, colonies per centimeter of root were constant throughout the season. Colonization of roots of several other plants by *Verticillium* spp. was found to be similar to that observed with cotton. Colony densities on roots per unit of inoculum density in soil were fairly similar for most of the fungi. The one exception was *Rhizoctonia*, for which a comparatively much higher value was observed. Colony densities for the *Pythium* species and for *Rhizoctonia* were higher on roots with severe tissue damage than on roots exhibiting little or no damage. For the other fungi, colonies were equally abundant on roots with or without tissue damage.

Additional keywords: epidemiology, soilborne pathogens.

Investigations on root-pathogen interaction of cotton (*Gossypium hirsutum* L.) plants and *Verticillium dahliae* Kleb. showed that the fungus was primarily a rhizoplane or outer cortex colonizer (7,12). Studies with both greenhouse plants growing in naturally infested soil (7) and field-grown plants (12) yielded similar results. Colony densities of *V. dahliae* on roots were directly proportional to soil inoculum densities. The fungal colonies were small, localized, and scattered randomly along the root length. Colonization rates of roots were unaffected by soil moisture or soil temperature (12).

Comparable quantitative information on colonization of plant roots by other fungi under field conditions is not available, nor is much known about the various factors affecting colonization of roots by fungi. To broaden the knowledge of root-fungi interactions under field conditions and to provide comparative data so as to better assess the findings on the interactions of roots with *V. dahliae*, a study of some of the variables affecting colonization of cotton roots by several fungi was undertaken. The fungi included in this study were *V. dahliae*, *V. tricorpus* Isaac, *Pythium ultimum* Trow, *P. aphanidermatum* (Edson) Fitzp., an unidentified *Pythium* species, *Rhizoctonia* species, a *Gliocladium* species, and two *Trichoderma* species.

MATERIALS AND METHODS

Field plot and root isolation. The studies on colonization of roots presented here were conducted simultaneously with and used the same plated roots of the study on colonization of cotton roots by *V. dahliae* and *V. tricorpus* in an accompanying paper (12). Field plot design, root sampling, isolation, and plating methods were those reported there. Briefly, a 1.6-ha plot, primarily designed to study the influence of inoculum density on disease incidence and yield (1), was established in 1975 at the University of California West Side Field Station in the San Joaquin Valley by incorporation into the soil of tomato (*Lycopersicon esculentum*

Mill.) debris naturally infested with *V. dahliae*. In 1975, both tomato and cotton were grown in replicated plots, whereas in 1976 and 1977 cotton was replanted on the same plots. Soil samples for isolating fresh roots were collected with a 2-cm-diameter soil tube to a depth of 30 cm at biweekly intervals from three replicate blocks with the highest inoculum density. Root segments were retrieved from the samples by a wet sieving technique and plated on a cellophane extract-pectate agar medium. When desired, before plating, freshly washed roots were segregated into various categories on the basis of physical appearance. Generally, three categories were used: white (roots with no or minor apparent root or cellular damage), brown (extensive browning on the surface or necrotic local lesions), and severe damage (extensive cortical collapse or a brown stele, some appeared dead).

The ratio between colony density on roots exhibiting extensive browning and/or cortical tissue damage and those exhibiting little or no tissue damage was calculated for the different fungi. For the last two samplings of 1977, when recently killed roots (complete collapse of cortex and loss of rigidity but with still recognizable root tissue) were plated, the ratio between these roots and those exhibiting little or no damage was also calculated. Values much greater than six for these ratios have been recorded as <6 since they resulted from calculating a ratio in which one of the numbers was small and statistically less meaningful.

Additional plant root samplings. Roots of several weed species growing on the plots at the West Side Field Station were collected in the early spring of 1977 and examined for colonization by *Verticillium* spp. The plants, growing in the alleys of field plots, examined were: tomato, lamb's-quarter (*Chenopodium album* L.), sow thistle (*Sonchus oleraceus* L.), and wild turnip (*Brassica campestris* L.). At the same time, alfalfa (*Medicago sativa* L.), which had been planted the preceding fall in a plot known to have a high level of *V. dahliae* (1), was also examined. Similarly, one commercial cotton field was sampled in September of 1976 and another commercial cotton field and a pistachio orchard were sampled in September of 1977. In 1983, colonization of cotton roots was assessed at three sampling dates during July and August in a heavily infested (*V. dahliae*) plot located nearby the 1977 field plots.

Soil inoculum densities. Inoculum densities of *V. dahliae* and *V. tricorpus* in soil were determined as previously (12,13). Populations of *P. ultimum* in soil were determined by the soil-drop method of Stanghellini and Hancock (17) and those of *P. aphanidermatum* by the procedure of Burr and Stanghellini (5). The soil populations of *Rhizoctonia* were determined by the wet sieving technique of Weinhold (18). A slight modification, consisting of soaking samples of soil (100 g) in sodium hexametaphosphate (1%) for 20 min before the sieving was introduced after separate tests had established that the sodium hexametaphosphate did not affect the counts of *R. solani*.

Populations of the *Gliocladium* species and the *Trichoderma* species in soil were determined by dilution plating and by a wet sieving technique similar to that used for *V. dahliae* (2,13). In the dilution assay, appropriate dilutions of air-dried soil in sterile water were plated on the cellophane pectate-Tergitol medium used for the root platings. In the wet-sieving assay, air-dried soil (1 g) was suspended in sodium hexametaphosphate (1%) for 20 min. The suspension was then washed with running tap water through a series of soil sieves, and the residues retained on the 124-μm-mesh (the top sieve), 38-μm-mesh, and 20-μm-mesh sieves were collected and plated on the above medium.

The soils used in the assays for inoculum density of the various fungi were collected in the same plots and at the same time as the soil samples used for the fresh root isolations. Three separate plots were assayed for the various fungi on each of three sampling dates in 1977 (either the 24 May or 16 June sampling, the 9 August sampling, and either the 2 or 16 September sampling). All soil weights are expressed on the basis of air-dried soil that had 3.5% moisture based on oven-dried weight.

Identification and pathogenicity tests. *Pythium* species were identified on the basis of sporangium and oospore formation on the cornmeal agar-water culture (10,16). The pathogenicity of isolates of *Rhizoctonia*, as measured by the maceration of cotton seedling hypocotyls, was determined by the procedure of Weinhold et al (19).

RESULTS

Fungal growth from plated roots. Numerous fungi grew from the roots as randomly scattered localized colonies. Each fungal colony appeared to occupy 3–5 mm of root. *Pythium* species grew most rapidly (radial extension) of all the fungi present, and their growth patterns were similar to those observed for *Pythiaceous* fungi on water agar. Initially, *Rhizoctonia* species grew rapidly as an unbranched hypha ($\pm$5 mm), but then growth was restricted. Colony morphology was distinctive and distinguishable from other fungi. Populations of three fungi, two *Trichoderma* spp., and one *Gliocladium* sp. were followed because of their common occurrence on roots and their distinctive colony morphology on the medium.

In the isolation of *Rhizoctonia* from the root assay plates, an occasional *Fusarium* sp. was encountered. For comparative purposes, roots (14 July sampling) were plated on Komada's medium (15) selective for *Fusaria*. For the various *Fusarium* species that grew from the roots, colony size and the pattern of distribution on roots were similar to those observed for *V. dahliae* and other fungi.

Identification and pathogenicity tests. Hyphal tips were taken from all colonies of *Pythium* and *Rhizoctonia* within 2–4 days after plating and transferred to potato-dextrose agar for verification of identity. Three *Pythium* spp. accounted for 148 of 151 isolations of *Pythium*: *P. ultimum*, *P. aphanidermatum*, and an unidentified *Pythium* species. The unidentified *Pythium* species was a sphaerosporangiate type (sporangia 26–28 μm, slightly larger than those of *P. ultimum*), and only the asexual stage was produced in the oatmeal-cholesterol (0.005%)-water culture. Several isolates were examined by R. G. Pratt (USDA, Mississippi State, MS) and were identified as isolates with vegetative hyphal bodies and growth rates similar to those of *P. sylvaticum* (Campbell & Hendrix).

Only a portion of the isolates of *Rhizoctonia* proved pathogenic to cotton. However, the fraction of isolates obtained from roots that were pathogenic (nine of 13 tested) was similar to that for isolates obtained from the soil (five of seven tested). Whereas the pathogenic isolates induced maceration lesions on cotton hypocotyls within 36 hr, the other isolates only induced a mild brown streaking. The pathogenic isolates were identical in growth characterisitics, colony morphology, and in their effect on cotton seedlings with authentic *R. solani* Kühn AG-4 (19).

Seasonal effects on root colonization. Colonization of cotton roots by the *Pythium* species was measurable throughout the growing season (Fig. 1). The total population counts (and standard error) observed during June 1977 (not included in Fig. 1 as a breakdown into separate *Pythium* species was not made) were in the same range (10 ± 3 [10^{-3}] and 17 ± 4 [10^{-3}] colonies per cm root on 19 May and 16 June, respectively) as those observed later in the season. Colony density was quite cyclical and was closely correlated with soil moisture (Fig. 1). Highest density of colonies occurred immediately after an irrigation and then dropped with time thereafter. All three *Pythium* species exhibited a similar colonization pattern.

In contrast to the *Pythium* species, colonization of cotton roots by *Rhizoctonia*, *Trichoderma*, and *Gliocladium* species was relatively constant with time (Fig. 2). Colonies of the latter fungi were localized (colony length on root was <4 mm), randomly scattered along the root length, and were quite sensitive to brief sodium hypochlorite treatment. A 5–15-sec exposure to 0.5% sodium hypochlorite reduced colony number by half, and a 3-min exposure eliminated nearly all colonies.

Colonization in relation to inoculum density. The values for the populations for each of the fungi in soil (Table 1) did not differ significantly among the three field plots or the three sampling dates tested. The population density of the unidentified *Pythium* species was determined simultaneously with that of *P. ultimum* in the

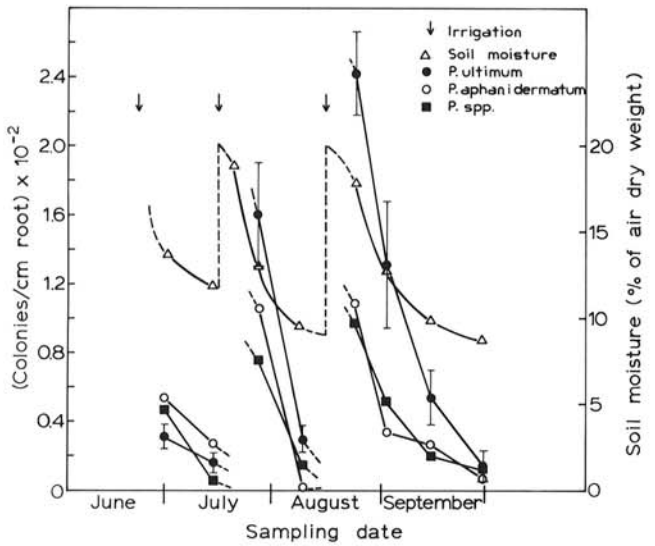

Fig. 1. Soil moisture and colony densities on cotton roots for three *Pythium* species as a function of time. The standard error for *P. ultimum* is indicated with vertical lines. The standard error for the other *Pythium* species were similar.

soil-drop assay. This *Pythium* species grew slower and had a slightly greater branching frequency than *P. ultimum* in this assay. Over a series of experiments involving the plating of a total of about 20 g of soil, the soil assay for *P. aphanidermatum* yielded only one positive colony. Either the soil population of *P. aphanidermatum* was extremely low or the soil assay did not function well with the panoche clay loam soil of the West Side Field Station.

The colonization rate (colonies, cm^{-1} root, $propagules^{-1}$, g soil) of roots, based on the observed inoculum densities, were similar for most fungi, being within an order of magnitude of 10^{-3} colonies per centimeter of root, propagule per gram of soil (Table 1). The one exception was *Rhizoctonia*, which had a colonization rate more than 100 times higher than the other fungi.

Colonization in relation to host species. Both *V. dahliae* and *V. tricorpus* readily colonized roots of other plants with localized, randomly spaced colonies as observed with cotton (12). The colonization values found for roots of these plants, along with those found for pistachio roots and for cotton roots in commercial fields, were remarkably similar, being around $2(10^{-3})$ and $10(10^{-3})$ colonies, μm^{-1} root, microsclerotia^{-1}, g soil for *V. dahliae* and *V. tricorpus*, respectively (Table 2).

Colonization rates varied both between plants for the *Verticillium species* and between *Verticillium* species for a given plant (Table 2). For most plants, *V. tricorpus* was a more effective colonizer of roots (fourfold or more) than *V. dahliae*. The only observed exception was tomato, for which *V. dahliae* was a more

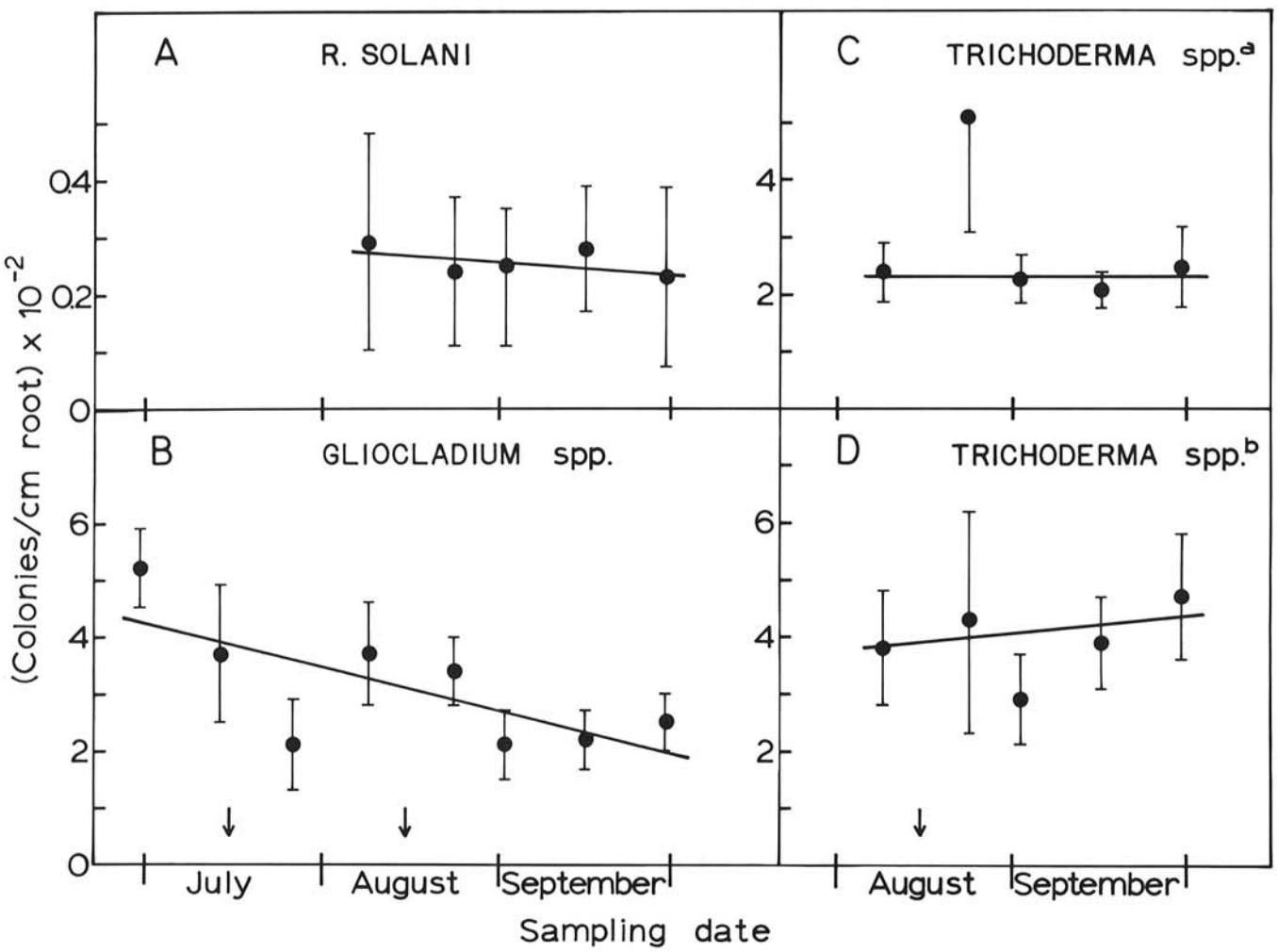

Fig. 2. Colony densities on cotton roots for four fungal species as a function of time. Arrows indicate irrigations. The standard error is indicated with vertical lines. Regression lines and equations were obtained with the least squares method. With *Tricodermia* spp. A, the single unusually high value was omitted from the regression analysis. The regression equation for the *Gliocladium* sp. $y = 6.0(10^{-2}) - 2.4(10^{-4})$(postplant days) was significant at $P = 0.05$. For the other regression lines, the slopes were not significantly different from zero at $P = 0.05$.

effective (two times) colonizer than *V. tricorpus*. There was no consistent pattern for the *Verticillium* species in their relative colonization of plant species. For example, *V. dahliae* colonized tomato roots four times more effectively than cotton roots, while *V. tricorpus* was only one-third as effective on tomato roots as on cotton roots (Table 2).

TABLE 1. Inoculum densities and colonization of cotton roots by fungi per unit root length and per unit inoculum density in soils for 1977

Isolation method and organism	Propagules (g^{-1} soil)[a]	Colony density[b] ($\times 10^{-3}$)
Soil drop		
P. ultimum	83 ± 11	0.3
Pythium sp.	6.0 ± 1.7	1.7
Wet sieving		
Verticillium dahliae[c]		2.0
V. tricorpus[c]		5.8
Rhizoctonia spp.	0.004 ± 0.001	620
Gliocladium spp.	52 ± 13	0.7
Trichoderma spp. A	42 ± 4	0.6
Trichoderma spp. B	71 ± 14	0.5
Soil dilution		
Gliocladium spp.	420 ± 90	0.08
Trichoderma spp. A	680 ± 93	0.04
Trichoderma spp. B	1,230 ± 190	0.03

[a] Values reported include standard errors and means for all assays since no significant differences were found among the three field blocks or among the three assay dates (either 24 May or 16 June, 9 August, and either 2 or 16 September).

[b] Colonies, cm^{-1} root, $propagules^{-1}$, g soil. Actual values in the table should be multiplied by 10^{-3}. Values reported are based on mean colonization values (colonies per centimeter of root) for the season for fungi exhibiting minor seasonal variations and on the highest colonization value observed for fungi (*V. tricorpus* and *Pythium*) that exhibited marked variations during the season. Except for the *Verticillium* spp., values were not corrected for colony splitting by random root breaks (12). If mean colony size for the *Pythium* spp. was about 30 mm (see text), then reported values should be multiplied by 0.3. If mean colony size for the other species was the same as that of the *Verticillium* spp., then reported values should be multiplied by 0.83.

[c] Values taken from Huisman (12), which were based on the data taken during 1977 from the same roots (and soil) as the other fungi in the table.

TABLE 2. Colony densities for *Verticillium* on roots per unit inoculum density in soils for several plants

Locations and crops	Colony density ($\times 10^{-3}$)[a]		Ratio tric/dahl
	V. dahliae	*V. tricorpus*	
WSFS[b]			
Cotton, 1976	1.7	14	8.2
Cotton, 1977	2.0	5.8	2.9
Cotton, 1983	1.7	nd[c]	···
Tomato, 1976	11.4	4.2	0.4
Tomato, 1977	6.4	nd (<7)	···
Sow thistle	2.2	14	6.4
Wild turnip	0.5	23	46
Lambs'-quarter	1.1	5.3	4.8
Alfalfa	1.7	nd (<0.3)	···
Other locations[d]			
Cotton A, 1976	1.3	nd	
Cotton B, 1977	2.5	nd	
Pistachio, 1977	0.8	nd	

[a] Colonies, cm^{-1} root, $microsclerotia^{-1}$, g soil. Actual values in the table should be multiplied by 10^{-3}. The values in this table were corrected for colony splitting due to random root breakage as described in Huisman (12). Figures in brackets refer to the lower limit of detection relative to the measured inoculum density of *V. tricorpus*. Where no lower limit of detection is indicated, *V. tricorpus* was not detectable in the soil assay.

[b] Data are based on root samples collected at the West Side Field Station. The 1976 field data were obtained from roots collected in September. The 1983 data were obtained from roots collected during July and August. The remaining entries were based on data obtained from roots collected on 19 May 1977.

[c] nd = No colonization detected.

[d] Data are based on root samples collected during September in commercial cotton fields and a pistachio orchard with a cotton cropping history.

Association of fungi with tissue damage. For most fungi, apparent cortical or stelar damage had no influence on colony density on roots of cotton. No significant differences ($P = 0.05$) were found in colony densities for either of the *Verticillium* spp. among any of the categories of root health ranging from clean white to extensive collapse of the cortex for each of nine sampling dates (27 September 1976 and samplings from 30 June 1977 onward). The ratio between colony density on roots exhibiting extensive browning and/or cortical tissue damage and those exhibiting little or no tissue damage did not deviate significantly from unity (1.0) for nearly all of the sampling dates for the two *Verticillium* spp., the *Gliocladium* species, and the *Trichoderma* species (Fig. 3).

With *Pythium* species, the above ratio was always greater than one (Fig. 3). Because little difference was noted among the three *Pythium* species with respect to root colonization, the data for them were treated collectively. Except for the first irrigation, when colony densities were low, the ratio had the lowest value immediately after an irrigation when the largest colony densities occurred. With time, the ratio at first increased, as a greater proportion of the remaining colonies occurred on the more damaged roots, but subsequently this ratio dropped again. However, most of the *Pythium* colonies at that time were associated with dead roots (Fig. 3). Most of the colonies of *Pythium* in the damaged roots were associated with a brown stele.

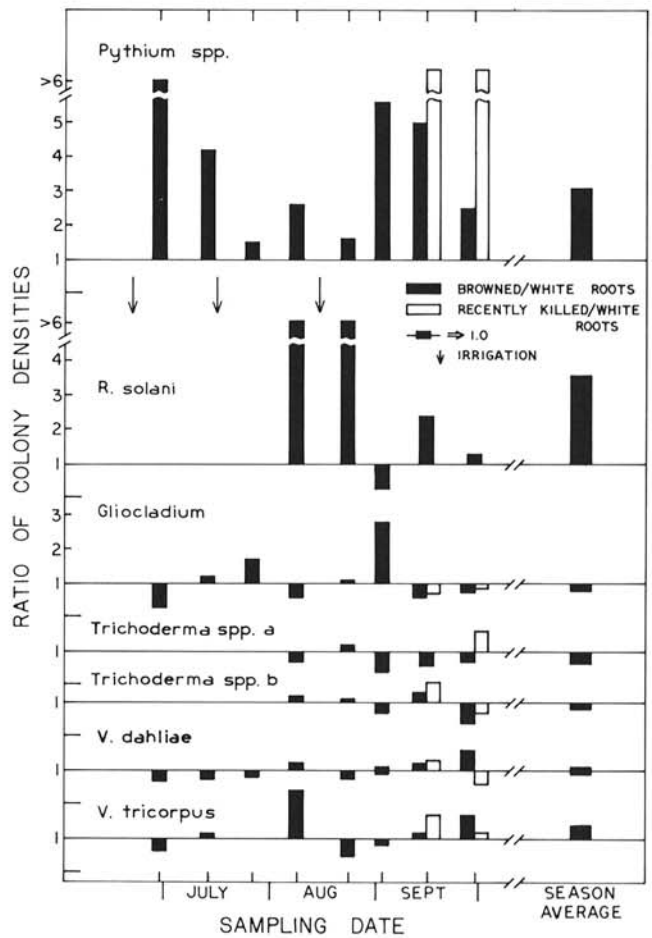

Fig. 3. Ratios of colony densities on roots showing extensive cortical or stelar tissue damage to the colony densities on roots exhibiting little or no tissue damage for the indicated fungi on eight sampling dates. The ratios are plotted relative to the 1.0 value, thus emphasizing deviations from unity. Sampling dates are indicated by small lines at the base of the graph. Data for the *Pythium* spp. are the combined results for the three *Pythium* species described in the text.

In such roots, the vascular bundle was visible as a brown outline suggestive of cellular damage and subsequent oxidation in or near the stelar tissue. The brown stele usually extended the full length of the root segment including segments as long as 3 cm. The cortical tissue on such roots exhibited little or no damage.

Rhizoctonia also was found to be preferentially associated with roots exhibiting a high incidence of tissue damage (Fig. 3). The ratios (Fig. 3) for this fungus are subject to considerable statistical error since they are based on very low numbers (four to six colonies/sampling date). The average ratio for the season (based on 24 colonies) is a more reliable measure of the association with damaged roots, since the colonization of roots was relatively constant with time (Fig. 2A).

Total populations of fungi on roots. Total number of fungi growing from roots averaged 1.3 colonies per centimeter of root. The colony density did not differ significantly among the various categories of cortical damage (Table 3). Older roots (large diameter) and younger roots (with tips) also did not differ significantly from other roots in colony density (Table 3). The number and diversity of fungal colonies growing from recently killed roots did not differ much from that observed on living roots. Roots obtained during September from a commercial cotton field had total colony densities similar to those from the West Side Station (Table 3).

Colony size of *Pythium* on roots. When *Pythium* spp. grew from root segments, individual hyphae usually emerged simultaneously from along the entire 1–2-cm root segment, suggestive of a large colony size. In the 16 September plating of long root segments, six colonies of *Pythium* were observed: Two of these grew from the entire length of the root piece (35 and 50 mm in length); three grew from large segments of plated pieces, with the colony beginning at one end of the segments (the length of the colony relative to the root segment length being, in millimeters, 20–46, 15–40, and 30–100); and one grew as a small colony (9 mm) in the middle of a root piece (50 mm). Application of the split colony method (12) for estimating length of colonies to these limited data suggests an average colony length of over 30 mm.

DISCUSSION

Both *Verticillium* species readily colonized roots of a variety of plants in the field (Table 2). The findings are consistent with reports on colonization of diverse plant roots by *V. dahliae* under greenhouse conditions. Evans et al (7,8) showed that *V. dahliae* colonized the rhizoplane of a large number of greenhouse-grown plants, with colony numbers being proportional to soil inoculation density. Results very similar to those of Evans (7,8) were obtained by Benson and Ashworth (3) with a number of *Verticillium*-immune crops using field soil under greenhouse conditions. The latter also reported that roots collected from barley growing in *Verticillium*-infested fields exhibited localized rhizoplane colonization by this fungus analogous to that observed in the greenhouse studies.

TABLE 3. Colony densities of total fungi on cotton roots

Root type[a]	Colonies, cm^{-1} root, and standard error ($\times 10^{-2}$) on sampling date (1977)			
	14 July	2 Sept	27 Sept[b]	30 Sept
White	151 ± 10[c]	89 ± 8	122 ± 12	111 ± 10
Brown	156 ± 8	94 ± 5	137 ± 7	137 ± 7
Tips	120 ±28	nd[d]	nd	nd
Large diameter	nd	102 ± 10	136 ± 5	nd
Severe damage	176 ± 49	93 ± 10	144 ± 6	133 ± 7

[a] Root segments were separated on the basis of apparent cortical damage (white, brown, severe damage—see Methods), the presence of root tips (tips) or diameters two- to fivefold larger (large diameter) than the normal feeder roots, most had undergone secondary expansion.

[b] The 27 September sampling was collected from a commercial cotton field and consisted of three subsamples collected separately. The other three samplings were from the West Side Field Station.

[c] In all cases no significant differences ($P = 0.05$) were found for the colonization values among the root categories for a given sampling date.

[d] nd = Not done.

Distinct differences in the colonization frequency of roots occurred for a given *Verticillium* species among the plants (Table 2). Evans and Gleeson (7), in their greenhouse studies, also observed differences in the colonization rate of roots by *V. dahliae* among the plant species examined. The colonization frequencies of *V. dahliae* and *V. tricorpus* did not change in the same proportion among the plant species (Table 2). Thus, the colonization frequency of roots would appear to be a unique property of each plant species-fungal species interaction. The observed colonization of cotton roots by *V. dahliae* in commercial fields was in good agreement with that observed in the plots at the West Side Field Station (Table 2) and suggests the results are broadly applicable to field conditions.

V. dahliae colonized plant roots with no evident damage to root tissue. The ratio of colony density on damaged roots to that on apparently healthy roots should be unity if there is no preferential association of the fungus with visible tissue damage and in excess of unity if there is. For *V. dahliae*, this ratio did not deviate significantly from one for any of the sampling dates (Table 3, Fig. 3). The various forms of tissue damage observed on the roots thus cannot be ascribed to *V. dahliae*.

The combined information presented here and that cited above lead to the following conclusion. *V. dahliae* is primarily a colonizer of the rhizoplane and/or root cortex and is adapted to a wide host range. Almost all the plants thus far examined (57 out of 63 species), including both dicotyledenous and monocotyledenous plants, were colonized (3,6–8). The colonization rate of roots is surprisingly uniform. Evans et al (8) found only a 10-fold difference between the most colonized (thorn apple) and the least colonized (wheat) plants. They also found that relative colony size on the roots of a number of plants was similar. Three separate studies yielded colony densities (colonies, μm^{-1} root, microsclerotia^{-1}, g soil) of 0.4–4.6 (10^{-3}) (3, calculated from data presented), 1–10 (10^{-3}) (7,8, given and estimated from data presented), and 0.5–11 (10^{-3}) (Table 2). Although these values represent real differences among plant species, and probably also differences between soil types, the spread (20-fold) is only a little over one order of magnitude. Whether a plant is susceptible or immune to systemic invasion appears unrelated to the ability of the fungus to colonize the root cortex (8). The principal difference between wilt susceptible and immune plants would appear to be the ability by the pathogen to systematically invade the vascular system of the former. However, systemic invasion is of profound significance in the buildup of populations in soil (14).

Based on the information presented here, soilborne inoculum of *V. tricorpus* appears to interact with plant roots in a similar manner to that of *V. dahliae*. The only observed difference was that *V. tricorpus* was a more effective root colonizer on most plants than *V. dahliae* (Table 2).

The estimation of the colony density of *Pythium* on roots is subject to considerable error. With the mean 12-mm length of plated root pieces, the large colony size would result in an overestimation of colony densities. In contrast to this, the lack of uniform colonization with time (Fig. 1) would result in an underestimation of colony density since the plated roots represent root growth over an extended time period. Comparisons between *Pythium* species and sampling dates, however, would not be affected, since the errors are common to all reported values.

P. ultimum, P. aphanidermatum, and the *Pythium* species all had similar root colonization patterns. No pronounced increase in *P. aphanidermatum* relative to *P. ultimum* was observed during the hot summer months (Fig. 1). Such a relative increase might be expected in view of the high temperature tolerance of *P. aphanidermatum* in comparision with *P. ultimum* (16). The soil temperature data, however, were consistent with the above results. With the exception of the top few centimeters, soil temperatures seldom were above 28 C (12).

Colonization of roots by *Pythium* differed from that of other fungi. In contrast to the small localized nature of colonies of *Verticillium* (colony length of 2–3 mm) and other fungi, colonies of

Pythium were about an order of magnitude longer. The frequent association of these colonies of *Pythium* with a brown stele is suggestive that after initial contact with the root, these fungi move primarily in the vascular bundles of the root. The *Pythium* species were not tested for pathogenicity. However, Hancock (10) reported that all tested isolates of *P. ultimum* obtained directly from soil collected at or near the West Side Field Station were pathogenic (preemergence damping-off) on cotton. Much of the colonization of roots by *Pythium* species apparently occurred during a short period of high soil moisture immediately after irrigation. This phenomenon is consistent with the well-known high moisture requirements of Pythiaceous fungi (11). At the time of the initial root colonization, no root damage was apparent as these fungi were found with almost equal frequency on white as on severely browned roots (Fig. 3). Subsequently, colony densities on roots dropped dramatically as a result of dilution by new root growth and by a shift of remaining colonies into the brown stele roots and finally into dead roots. The above pattern, along with the unusually long length of the colonies of *Pythium* and the known ability of *P. ultimum* and *P. aphanidermatum* to invade and kill roots, are indicative that the *Pythium* species were involved in root death. If such were the case, the data can be interpreted as representing the process of initial colonization of healthy roots followed by cellular damage and death (brown stele roots) and the final collapse of tissue structure resulting in their classification as dead roots. The pattern observed after the first irrigation (Fig. 3). appears to be inconsistent with the above analysis. However, the low colony densities observed on the first assay date may indicate that the peak colonization period had already passed. Additional work will be needed to fully validate the interpretation.

The colonies of *Rhizoctonia* tended to occur more frequently on roots showing extensive browning and cortical collapse than on other roots (Fig. 3). This is consistent with the ability of *Rhizoctonia*, especially *R. solani*, to induce cortical lesions.

The colonization rate of roots by *Rhizoctonia* was exceptionally high, being more than two orders of magnitude higher than that of the other fungi (Table 3). This high value is probably not due to an underestimation of populations in soil. The value of 0.4 propagules/100 g of soil is within the range of inoculum densities observed in the San Joaquin Valley (20). Additionally, Welch (20) found that introduction into test soil of populations of *R. solani* equivalent to those found by the wet sieving assay in natural field soil yielded disease incidence on cotton seedlings equivalent to that observed in the field soil.

The incidence of tissue damage, both cortical and stelar, observed on roots was much higher than could be accounted for by the incidence of either the *Pythium* species or *Rhizoctonia* on roots. At best, *Pythium* species were isolated from less than 10% of the roots exhibiting stelar browning, and the incidence of *Rhizoctonia* in the browned root category was considerably less than this. Such low values suggest that other organisms were probably responsible for much of the observed root damage. The determination of the relative colonization frequency of damaged to undamaged roots should prove to be a useful first step in identifying potential pathogens of the root cortex.

The colonization rate of roots by the *Gliocladium* and *Trichoderma* species can only be taken as approximate. Both methods for estimating populations in soil (soil dilution and wet sieving) have their shortcomings. Because these fungi sporulate readily, the soil dilution assay probably overestimated their effective population density by dispersing spore clusters. The wet sieving assay only measured the population of colonized organic debris larger than 20 μm and necessarily missed smaller propagules. The latter assay is probably closer to the effective soil population than the soil dilution assay. Soil organic debris has been postulated to be the major source of inoculum for root colonizing microbes (4,9).

The colonization of cotton roots by saprophytic fungi (*Gliocladium*, *Trichoderma*, and *Fusarium* species) was, in most respects, identical to that reported for *V. dahliae*. Colonization rates were nearly constant during the season and apparently not greatly affected by soil moisture or soil temperature (Fig. 2; pertinent soil temperature data are given in Fig. 3 in 12). Colony densities on roots for the *Gliocladium* species and the *Trichoderma* species (using soil populations estimated by the wet sieving method) were also fairly similar to those found for *V. dahliae* (Table 1). The nearly equivalent densities on the various root categories for *V. tricorpus*, *Gliocladium* species, and *Trichoderma* species (Fig. 3) suggest that these fungi, as *V. dahliae*, colonize plant roots with no evident damage.

The data presented here suggest that root colonization by fungi is an early event in the life of roots. The nearly equivalent total colony density of fungi on young roots compared with that of older roots (Table 3) is consistent with this interpretation. With colonization occurring only in the early stages of the life of the roots (near the root tip), a constant colony density would be expected that would be little influenced by root health, root age, or plant age (Figs. 2 and 3).

The finding that root colonization by *Gliocladium* and *Trichoderma* was analogous to that reported for *V. dahliae* and *V. tricorpus* support the interpretation that *Verticillium* is primarily a colonizer of rhizoplane or cortex much like other saprophytic fungi. The observation that the various fungi growing from roots were randomly scattered along the root as small, localized colonies further supports the conclusion that the colonization parameters observed for *Verticillium* (12) probably apply to most fungi that colonize roots.

LITERATURE CITED

1. Ashworth, L. J., Jr., Huisman, O. C., Harper, D. M., Stromberg, L. K., and Bassett, D. 1979. Verticillium wilt disease of cotton: Influence of inoculum density in the field. Phytopathology 69:483-489.
2. Ashworth, L. J., Jr., Waters, J. E., George, A. G., and McCutcheon, O. D. 1972. Assessment of microsclerotia of *Verticillium albo-atrum* in field soils. Phytopathology 62:715-719.
3. Benson, D. M., and Ashworth, L. J., Jr. 1976. Survival of *Verticillium albo-atrum* on nonsuscept roots and residues in field soil. Phytopathology 66:883-887.
4. Bowen, G. D., and Rovira, A. D. 1976. Microbial colonization of plant roots. Annu. Rev. Phytopathol. 14:121-144.
5. Burr, T. J., and Stanghellini, M. E. 1973. Progagule nature and density of *Pythium aphanidermatum* in field soil. Phytopathology 63:1499-1501.
6. Evans, G. 1971. Influence of weed hosts on the ecology of *Verticillium dahliae* in newly cultivated areas of the Namoi Valley, New South Wales. Ann. Appl. Biol. 67:169-175.
7. Evans, G., and Gleeson, A. C. 1973. Observations on the origin and nature of *Verticillium dahliae* colonizing plant roots. Aust. J. Biol. Sci. 26:151-161.
8. Evans, G., McKeen, C. D., and Gleeson, A. C. 1974. A quantitative bioassay for determining low numbers of microsclerotia of *Verticillium dahliae* in field soils. Can. J. Microbiol. 20:119-124.
9. Gray, T. R. G., Baxby, P., Hill, I. R., and Goodfellow, M. 1967. Direct observation of bacteria in soil. Pages 171-197 in: The Ecology of Soil Bacteria. T. R. G. Gray and D. Parkinson, eds. Liverpool Press, Liverpool. 681 pp.
10. Hancock, J. G. 1977. Factors affecting soil populations of *Pythium ultimum* in the San Joaquin Valley of California. Hilgardia 45:107-122.
11. Hendrix, F. F., and Campbell, W. A. 1973. Pythiums as plant pathogens. Annu. Rev. Phytopathol. 11:77-98.
12. Huisman, O. C. 1988. Seasonal colonization of roots of field-grown cotton by *Verticillium dahliae* and *Verticillium tricorpus*. Phytopathology 78:708-716.
13. Huisman, O. C., and Ashworth, L. J., Jr. 1974. Quantitative assessment of *Verticillium albo-atrum* in field soils: Procedural and substrate improvements. Phytopathology 64:1043-1044.
14. Huisman, O. C., and Ashworth, L. J., Jr. 1976. Influence of crop rotation on survival of *Verticillium albo-atrum* in soil. Phytopathology 66:978-981.
15. Komada, H. 1976. A new selective medium for isolating *Fusarium* from natural soil. (Abstr.) Proc. Am. Phytopathol. Soc. 3:221.
16. Middleton, J. T. 1943. The taxonomy, host range, and geographical distribution of the genus *Pythium*. Mem. Torrey Bot. Club 20:1-171.
17. Stanghellini, M. E., and Hancock, J. G. 1970. A quantitative method for the isolation of *Pythium ultimum* from soil. Phytopathology 60:551-552.
18. Weinhold, A. R. 1977. Population of *Rhizoctonia solani* in agricultural

soils determined by a screening procedure. Phytopathology 67:566-569.
19. Weinhold, A. R., Bowman, T., and Dodman, R. L. 1969. Virulence of *Rhizoctonia solani* as affected by nutrition of the pathogen. Phytopathology 59:1601-1605.
20. Welch, L. L. 1976. Interactions of *Rhizoctonia solani* populations and propagule nutrition in agricultural soils. Ph.D. thesis. University of California, Berkeley. 84 pp.