Seasonal Colonization of Roots of Field-Grown Cotton by *Verticillium dahliae* and *V. tricorpus*

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ABSTRACT

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Colonization of roots of field-grown cotton plants by *Verticillium dahliae* and *V. tricorpus* was examined by plating washed roots on a growth restrictive medium. The roots were collected biweekly, starting when plants were in the first true leaf stage. Colonies of *Verticillium* readily developed from these roots. The density of colonies on roots correlated with the inoculum density of *Verticillium* in the soil. Colonization of roots by the two fungi continued throughout the growing season. Seasonal changes in soil moisture and temperature had no influence on the colonization rate of roots by *V. dahliae*, whereas colonization of roots by *V. tricorpus* was affected by soil temperature, being greater at 20–23 than at 28–31 C. The mean length of colonies on roots was relatively constant (2.3 mm) during the season. Early in the season, colonies were randomly distributed along the length of the root, but, from mid-July onward and coincident with measured increases in inoculum densities in soil, there was evidence for clustering of colonies along the root. Colonization of roots was not related to pathogenicity since *V. tricorpus* readily colonized both cotton and tomato roots but only infected the vascular system of tomato.

Additional keywords: epidemiology, soilborne pathogens.

Verticillium dahliae Kleb. has been shown to colonize the roots of a wide range of plant species, which include both those immune and susceptible to vascular infection (6–9,16). Extensive rhizoplane colonization of a number of plants by *Verticillium* under greenhouse conditions has been reported by several workers. Lacy and Horner (16) in greenhouse studies with artificially infested soils showed that *V. dahliae* could be recovered consistently from both immune and susceptible host roots. Evans et al (8) examined the roots of a wide range of young (20-day-old) plants grown in the greenhouse in naturally infested soil. They found colony density of *V. dahliae* on roots was directly related to inoculum density. Colonies were small, randomly scattered along the root surface, and probably confined to the rhizoplane since they were very sensitive to brief mercury treatment. Little information is available on the colonization of roots in the field. The purpose of this study was to determine the seasonal relationship between colonization of cotton roots (*Gossypium hirsutum* L.) by *V. dahliae* and *V. tricorpus* Isaac and inoculum density of the fungi in field soil.

MATERIALS AND METHODS

Field plots. The field plot at the University of California's West Side Field Station has been described and was primarily established for a field study on the influence of inoculum density on disease incidence and yield (2). Briefly, a 1.6-ha plot was established in 1975 with different quantities of tomato (*Lycopersicon esculentum* Mill.) debris naturally infested with *V. dahliae* to provide five replications of four different inoculum densities of the pathogen in the soil. In 1975, half of each block was planted to cotton (cultivar Acala SJ-2) and half to tomato (cultivar Early Pak-7). In 1976, the field was replanted to cotton but with four cultivars (SJ-2, SJ-4, SJ-5, and 70-110) as subplots of the original inoculum density plots. In 1977, the cultivars were replanted on their 1976 locations with the exception that the 70-110 cultivar was replaced by SJ-4.

Inoculum densities of *V. dahliae* and *V. tricorpus* in soil were determined by sieving and plating techniques (3,12) with results corrected for known underestimates of 10 and 45% for the fungi, respectively (2, *unpublished*). Assays were routinely performed in duplicate from a given soil sample. In preliminary experiments (*unpublished*), it was established that in soil, 5% of the population of *V. tricorpus* was present in the 20–38-μm fraction and 40% in the greater than 124-μm fraction. The larger size of microsclerotia of *V. tricorpus* accounted for the latter large value. On the polypectate media, colonies of *V. tricorpus* were readily distinguished from those of *V. dahliae* due to the larger, blacker, and more dispersed appearance of microsclerotia of the former.

Temperatures of soil at various depths and in the plant canopy (15 cm above the soil and in the center of the plant row) were monitored during a portion of the 1977 growing season with a four-channel recording thermograph. Air temperatures were obtained from the weather records maintained at the West Side Field Station.

Isolation medium. In the initial phases of the study, a pectate medium was used for assays of soil for *V. dahliae*. In later assays of soil and for the platings of roots, a cellophane extract-pectate medium was used, which facilitated the differentiation of *V. tricorpus* because of the production of a characteristic yellow pigment (21) by this fungus in the absence of intense microbial competition. The pectate medium (pH 7.0 ± 0.1) consisted of (per liter): 5 g of sodium polypectate or polygalacturonic acid (neutralized with NaOH); 5 ml of 2 M KNO_3; 10 ml of 1 M $KHPO_4$, pH 7.0; 0.5 ml of 2 M $MgSO_4$; 10 ml of 100 ppm biotin; 1 ml of Tergitol NP-10; 15 g of agar; 250 mg of chloramphenicol; 200 mg of penicillin-G; 200 mg of chlortetracycline; and 200 mg of streptomycin sulfate. For the cellophane extract pectate medium, adapted from Taylor (21), the polypectate was reduced to 2 g, and a portion of the water was replaced by an extract obtained from autoclaving 20 g of untreated cellophane (PT-clear cellophane, Zellerbach Paper Co., San Francisco, CA) in water.

Root sampling, 1976. Soil samples used for isolating fresh roots were collected with a 2-cm-diameter soil tube to a depth of 30 cm. Care was taken to protect the samples from heat and desiccation during collection and transport. To keep the work load manageable, only a limited number of plots were sampled in the 1976 isolations of fresh roots. Inoculum densities used to relate densities of inoculum in soil to density of colonies of fungi on roots were those determined for each separate soil sample from which the roots were isolated. During 1976, inoculum density, infection data (2), and root density (*unpublished*) had exhibited no marked variation among the field replications. Most of the samplings of fresh roots were made during late September and October of 1976. In one experiment, soil samples for fresh root isolation and

inoculum density assays were collected to a depth of 120 cm, at 30-cm increments, in the plots with the highest inoculum. Many tomato volunteers occurred in the portion of the field with the 1975 tomato history. To obtain comparative data on colonization of roots by the *Verticillium* species, soil samples were collected beside tomato plants permitted to grow in the alleyways of the cotton subplots.

Root sampling, 1977. During the 1977 growing season, sampling of roots started when plants were in the first true leaf stage and continued thereafter at biweekly intervals. To keep the work load manageable, only three of the five replicates of the highest inoculum density plots were sampled in this fashion. The other two replicates and some plots with lower inoculum densities were sampled sporadically during the season. Because, in the 1976 assays, no differences were observed among the cotton cultivars for colony densities (colonies, cm^{-1} root, microsclerotia^{-1}, g soil) of the *Verticillium* species on roots, the cores of soil (0–30 cm depth) from the subplots (cultivars) were bulked into a 12-core composite sample. For studies requiring long (>2-cm) root segments, samples were collected (16 September) in the plots with high inoculum density with the aid of a spade and long root pieces were retrieved upon dispersal of the soil in buckets of water.

Separation of roots from soil. Soil containing roots was usually processed within 24 hr of collection. No significant differences were found in several trials among samples processed after storage at 4 C for 12, 24, or 48 hr after collection. The soil samples were suspended in 1% sodium hexametaphosphate and stored overnight at 4 C. The macroorganic fraction was recovered by the method of Torssell et al (22). Turbulence during the flotation step was kept to a minimum to minimize root abrasion by the soil mineral fraction. Miscellaneous debris was removed by various manipulations, including selective decanting, physical removal, and extensive washings of the debris containing root pieces onto a 1-mm-mesh sieve. The final root sample was suspended in water containing 1% sodium hexametaphosphate and 0.1% Tergitol NP-10, placed on a rotary shaker, and shaken at slow speed for 10–20 min. The washing was repeated two more times with thorough rinsing of the roots with tap water on a small 125-mm-mesh sieve between washings. The final suspension was rinsed in sterile water and collected with suction onto filter paper.

Plating of roots. With the aid of fine forceps and a dissecting microscope, roots were transferred to petri plates containing media and placed sequentially about 5 mm apart in seven rows such that each plate contained about 40 linear cm of root. For the 1976 experiments, the mean length of plated root pieces was 8 mm. During the 1977 platings, root pieces less than 10 mm in length (based on visual judgment) were avoided, and the mean length of plated roots was 12 mm. Forceps were dipped into alcohol, followed by sterile water between every five to 10 transfers. Care was taken to ensure that root segments transferred were free from miscellaneous debris. After 14–21 days of incubation at 25 C, plates were examined for colonies of *Verticillium* growing from root segments based on development of microsclerotia in the media, after most aerial growth of fungi had been removed by flooding the plates with ethanol followed by gentle washing (avoiding dislocation of roots) under running tap water.

Length of plated roots was initially obtained by direct measurement. This was accomplished by placing petri plates on 1-mm grid graph paper and estimating the length of each piece to the nearest millimeter. Because root pieces did not shrink significantly during the incubation period, it was also possible to use the random line-intersect method (18,22) for estimating the length of root pieces retrieved from the plates. A plot of estimates of root lengths obtained from the same sets of roots by the direct method against those obtained by the line-intersect method yielded a plot with a slope of 0.97 and 95% confidence limits for the slope of 1.04 to 0.91. The latter method was used for obtaining the greater majority of measurements of root length.

During the 1977 season, a total of about 1,500 cm of root segments was plated for each sampling date. In the calculation of colonies per centimeter of root, the number of colonies was corrected for colony splitting due to random breakage as described below.

Analysis of cross contamination. Because forceps were not sterilized between each root transfer, a possibility of cross contamination of root pieces handled successively existed. The possible occurrence of this phenomenon was evaluated statistically. The occurrence of *V. dahliae* on adjacent root segments (placed sequentially on agar) within rows of plated segments was noted whenever it occurred. Random probability was calculated (20) using the expression derived from the binomial distribution,

$$f_i = (N/k)[(k - (i + 1)q^i - 2(k - i)q^{i+1} + (k - i - 1)q^{i+2})].$$

In this expression, f_i represents the expected frequency, assuming random distribution, for one event (root segments positive for *V. dahliae*) occurring in strings of length i separated by one or more of the other events (root segments negative for *V. dahliae*) in samples taken k at a time ($k = 5$, the average number of root segments per plated row) for a total of N events (total root segments plated), and q represents the fraction of root pieces positive for *V. dahliae*. The G-statistic was calculated on the basis of f_i (the number of f_i positive root pieces associated with each class) where

$$\Sigma(i)f_i = \Sigma(i)\hat{f}_i = Nq.$$

Estimation of colony length on roots. The mean length of colonies of the *Verticillium* species on roots of cotton was estimated by using a statistical approach. Assuming that roots break randomly during the soil sampling and crumbling steps, the frequency at which a break would occur within a colony of *Verticillium* should be proportional to the fraction of the total root length covered by such colonies, i.e.:

$$B_c / B_t = L_c / L_t \quad (1)$$

where B_c is the number of root breaks occurring within a colony of *Verticillium*, B_t is the total root breaks (total root length/mean segment length), L_c is the root length covered by colonies of *Verticillium* (total colonies × mean colony length), and L_t is the total root length. Records were kept as to whether *Verticillium* emerged from the middle of a root piece (internal colony, i.e., colonies did not appear to touch either end of the plated root piece) or whether the region of root from which the colony of *Verticillium* emerged included the end of the plated root piece (terminal colony). Colonies with questionable locations (about 10%) were divided equally between the two categories. Because breakage of a root at the site of a colony of *Verticillium* would result in two root fragments, each with a colony at one end, the total colonies of *Verticillium* split by random breaks were taken as one-half of the observed terminal colonies. The equation (derived from expression 1) used for calculation of the colony length of fungi on roots was:

$$C = R((t/2)/(i + t/2)), \quad (2)$$

where C is the mean colony length, R is the mean root segment length, t is the number of observed terminal colonies, and i is the number of observed internal colonies. Root segments positive along their entire length for *Verticillium* were not included in the internal or terminal colony counts, as is required in this statistical formulation.

Without correcting for terminal colonies, colony densities determined from root plating will overestimate the actual number of colonies, with larger errors occurring with shorter root pieces. Plating of long root segments would minimize this error. The use of a soil corer for obtaining root samples limited the mean length of plated roots to approximately 1 cm. However, this disadvantage was offset by the ability to obtain a more random root sample from plots and ease of sampling. To correct for the error introduced by colony splitting, all values for colony density on roots reported here or used in calculations were adjusted as given in expression 3,

which was derived from expression 2:

$$\text{Net colonies} = \text{Number observed colonies} \times (R/(C+R)) \quad (3).$$

In this expression, the number of observed colonies represents the total colony count without regard to the terminal or internal location of the colonies on the root segments.

To evaluate the appropriateness of the above procedures, root segments of different mean lengths were plated from the same root lots on several sampling dates (24 August and 16 September). If the estimation procedure for colony length (expression 2) is appropriate, the calculated length of colonies on roots should be independent of the mean length of the plated root segments. If the correction factor (expression 3) is appropriate, colony densities on roots should be independent of mean segment length after correction but not before correction.

Distribution of colonies on roots. To permit an evaluation of the distribution pattern of colonies along the root length, records were kept of the number of root pieces plated and the number of root pieces giving rise to two or more of each of the *Verticillium* species. The data were analyzed for deviations from a random distribution as measured by the G-statistic.

Greenhouse studies. A small-scale study of the influence of temperature on colonization of cotton roots by the two *Verticillium* species was conducted in a greenhouse in the spring of 1977. A 5-cm layer of pea-size gravel was placed in 15-L closed-bottom containers. To facilitate bottom watering, a short piece of a 5-cm-diameter plastic pipe was placed vertically along one side, and the containers were then filled with field soil from the plot with the highest inoculum density of *V. dahliae*. Cotton plants (SJ-2), one per container, were grown at 25 C (day temperature; nighttime temperature was not strictly regulated and often dropped to around 20 C) for 3 wk, at which time three plants were transferred to each of four constant temperature rooms set at 20, 23, 28, and 31 C. Soil temperatures were monitored with thermometers inserted 15 cm into the soil and were read morning and evenings at 2- to 3-day intervals. Temperatures ranged ±2 C from the mean temperature indicated. Several plants were retained in the main greenhouse (25 C). At the end of 12 wk, root samples were recovered and handled as described for the field plots.

Systemic infection and pathogenicity tests. At the end of the 1976 growing season, cotton and tomato plants were evaluated for systemic infection. Stem segments (a 2-cm segment taken 5–10 cm above the soil line) were collected from 200 plants each of the SJ-2 and SJ-5 cotton cultivars and 100 of the volunteer tomato plants. Segments were surface sterilized, and a 0.5-cm slice plated on the cellophane extract-pectate media.

Forty random isolations of *V. dahliae* and 10 of *V. tricorpus* were made from localized colonies growing from segments of cotton root plated on media. The pathogenicity of these isolates was tested on 6- to 10-wk-old cotton plants (Acala SJ-2) grown in the greenhouse. Plants were inoculated by stem injection of 5–10 μl of a conidial suspension. Conidial suspensions were obtained by washing the surface of 7- to 14-day potato-dextrose agar slants of the isolates with 5 ml of sterile water. Disease incidence was assessed by evaluation of foliar wilt symptoms and vascular necrosis after 3–4 wk.

RESULTS

Inoculum densities in soil. In the fall of 1976, densities of *V. dahliae* (0–30 cm depth) ranged from 22.0 to 18 microsclerotia per gram of soil for the plots with the most and least inoculum. Corresponding values for *V. tricorpus* were 3.4 and 0.85. Over a 4-yr period involving numerous assays, the coefficient of variation (CV) within duplicate assays from the same soil sample ranged from 17 to 31% for *V. dahliae* and from 23 to 35% for *V. tricorpus*, while the CV between samples (after removal of sums of squares attributable to assay dates, field locations, and treatments) ranged from 36 to 57% for *V. dahliae* and from 35 to 70% for *V. tricorpus*. During the 1977 season, the respective values for the CV between samples were 46 and 54%.

Inoculum densities decreased dramatically with soil depth. For *V. dahliae*, observed densities in the high inoculum plots were 32.0, 7.9, 1.8, 1.5, and 0.9 microsclerotia per gram of soil for the 0–15-, 15–30-, 30–60-, 60–90-, and 90–120-cm depths, respectively. Corresponding values for *V. tricorpus* were 5.3, 1.5, 0.58, 0.13, and 0.04 microclerotia per gram of soil. Because most of the inoculum (90%) was located in the upper 30 cm of soil, almost all root samples used in these studies were collected from this soil layer.

Growth of fungi from plated roots. The roots obtained by the isolation procedure were free from soil particles or other debris. They consisted primarily of small diameter roots (about 0.3 mm diameter). Microsclerotia or other pigmented structures were never observed in or on live roots at the time of plating. *V. dahliae* and *V. tricorpus* grew from root pieces as localized colonies similar to other fungi.

The colonies of *Verticillium*, and those of other fungi, were quite sensitive to sodium hypochlorite. A 5–15-sec exposure to 0.5% hypocholorite reduced colony numbers by half, and a 3-min exposure eliminated nearly all colonies.

There was no evidence of cross contamination of successively handled root pieces during plating. In one experiment involving the plating of 795 root segments, of which 115 were positive for *V. dahliae*, there were 10 incidences of two adjacent pieces positive for *V. dahliae* and one incidence of three adjacent positive segments. The expected frequencies on the basis of random probability were, respectively, 10.6 and 1.4. During 1976 and 1977, in 15 separate experiments for which the frequency of adjacent root pieces was evaluated, the value of the G-statistic ranged from 0.1 to 14 with only three values exceeding the value of 3.8 required to indicate significant deviation ($P = 0.05$) from the expected random distribution.

For roots derived from the same sample, colony densities obtained from short segments differed significantly from those obtained from longer segments. However, after adjustment with the correction factor (see Methods), differences were no longer significant, suggesting the factor adequately adjusted for differences due to mean root length. In one experiment (24 August sampling), uncorrected and corrected (given in parenthesis) colony densities (colonies × 100 per centimeter of root) for short (mean 5.2 mm) and longer (mean 11 mm) root segments were, respectively, 3.6 (2.9) and 4.6 (3.0) for *V. dahliae* and 3.3 (2.4) and 2.7 (2.2) for *V. tricorpus*. With longer root segments (15–65 mm, 16 September sampling), there were no significant differences within or between uncorrected and corrected colony densities, although corrected values had a slightly lower coefficient of variation (5.4 vs. 7.2%). The latter finding probably reflects the fact that, with root segments much longer than the mean colony length, the correction factor approaches one and adjustments are minor.

Relation of colony density on roots to inoculum density in soil. The number of colonies of both *V. dahliae* and *V. tricorpus* per centimeter of cotton root was directly proportional to inoculum density in the soil (Fig. 1C and D). When colonization is expressed in terms of per unit inoculum density, colony densities colonies, cm^{-1} root, microsclerotia^{-1}, g soil for *V. tricorpus* (0.014) were about eight times higher than those for *V. dahliae* (0.0017). Part of the scatter observed in these figures is probably variability due to low total number of colonies counted, since most samples represented 200–400 cm of root examined. Because both variables in Figure 1 had significant and independent error components associated with their values, Bartlett's three-group method (20) was used for evaluation of the slopes and 95% confidence limits given in the figure. For tomato, a linear relationship between colony density on roots and inoculum density in soil was also observed (Fig. 1A and B).

Colonization of roots in relation to systemic infection and cultivars. Roots of the four cotton cultivars were evaluated separately for density of colonies of *Verticillium* species during the 1976 assays. No significant differences in colony density of *V. dahliae* or *V. tricorpus* on roots were observed among the four cultivars ranging from highly susceptible (70-100) to highly tolerant (SJ-5) to Verticillium wilt. Mean colony densities on roots of the four cultivars collected from plots with both high and low

inculum densities (18.8 and 3.2 microsclerotia per gram of soil, respectively, for *V. dahliae* and 3.8 and 0.9 for *V. tricorpus*) in two separate trials were 1.9 (range 1.6–2.5, CV = 20%) $\times 10^{-1}$ for *V. dahliae* and 13.4 (range 8.1–19.8, CV 30%) $\times 10^{-3}$ for *V. tricorpus*.

The relation between occurrence of systemic infection by *V. dahliae* and root colonization was examined by plating roots from soil samples taken from within 10 cm of clusters of severely infected plants (SJ-2, defoliated) and from clusters of apparently healthy plants (SJ-2, no vascular necrosis) located within the same inoculum density plot. No significant differences were observed in colony density on roots between these two categories. Mean colony densities, ranges, and coefficients of variation were essentially the same as those given above for the cotton cultivars.

Effect of other variables on colony densities on roots. Soil depth per se had no detectable influence on the colony density of the two *Verticillium* spp. on cotton roots. Roots obtained from lower depths (to 120 cm) in the soil profile had progressively lower colony counts but these were directly related to the lower inoculum density at those depths.

All the colonization data for the 1977 season for the two *Verticillium* spp. were subjected to an analysis of variance. The coefficients of variance for colonies per centimeter of root for *V. dahliae* and *V. tricorpus*, after removal of sums of squares attributable to assay dates, field location, and soil inoculum density, were 35 and 56%, respectively.

Soil temperature had a marked effect on the colonization of cotton roots by *V. tricorpus*. In greenhouse studies, no colonization of roots was observed at 31 C, and at 28 C colonization was barely detectable (only a single colony was observed) (Table 1). The greatest amount of colonization was observed at soil temperatures of 20 and 23 C. The roots were colonized by *V. dahliae* at all the temperatures with higher values at the lower temperatures although the differences between 20–23 C and 31 C were not significant (Table 1).

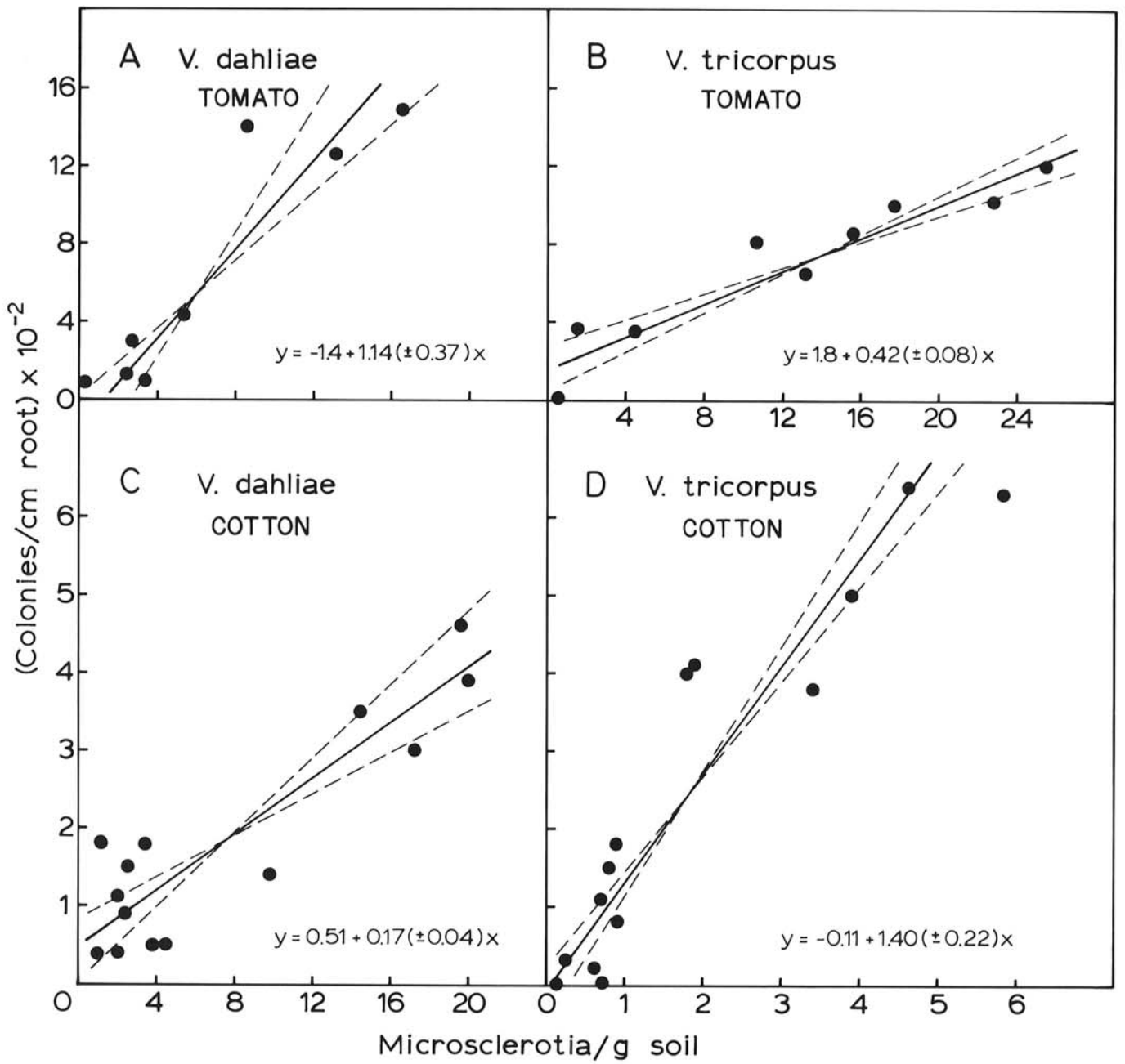


Fig. 1. Relationship between the inoculum density (microsclerotia per gram of soil) of *Verticillium dahliae* and *V. tricorpus* in soil and the observed colony density (colonies per centimeter of root) on cotton roots. Colony densities were corrected for colony splitting due to random root breakage as described in the text. The dashed lines and the value in brackets represent the 95% confidence limits of the slopes: **A,** *Verticillium dahliae* on tomato roots; **B,** *V. tricorpus* on tomato roots; **C,** *V. dahliae* on cotton roots; **D,** *V. tricorpus* on cotton roots.

Colonization of roots by the *Verticillium* species was similar for plants grown in the field and in the greenhouse. Roots retrieved from the field plot in June 1977 had 4.9 and 2.3 colonies per 100 cm for *V. dahliae* and *V. tricorpus*, respectively, whereas roots from 12-wk-old plants grown in the same soil in the 25 C greenhouse had 7.0 and 2.8 colonies per 100 cm, respectively.

Colony density on roots during the growing season. Colonization of cotton roots by *V. dahliae* had already occurred by the earliest sampling date (5 wk after planting, first fully expanded true leaf stage). *V. dahliae* exhibited a marked increase in colony density between June and August, but inoculum density also increased (from 25–70 microsclerotia per gram of soil) during this time period (Fig. 2A). Except for late June and early July, the colony density was essentially constant throughout the entire season (Fig. 2A). The lower values in late June and early July were related to the apparent lag between increases in inoculum densities in soils and corresponding increases in colony densities on roots. For this reason (see Discussion for more detailed rationale), the values for these dates were omitted in drawing a best fit regression line.

Roots of cotton were colonized by *V. tricorpus* throughout the growing season. However, the frequency of colonies on roots was not constant (Fig. 2B), with less colonization occurring during the summer months as compared with the early spring and the fall. Inoculum densities for *V. tricorpus* were essentially constant during the season (Fig. 2B). Consequently, changes in colony density paralleled the changes in colony frequency (colonies per centimeter of root) (Fig. 2B). The colony density observed toward the end of September 1977 (0.004, Fig. 2B) was only one-third that observed in October 1976 (0.014, Fig. 1D). Irrigations had no apparent effect on colonization of roots by the two *Verticillium* spp. (Fig. 2A and B).

Average soil temperatures at the West Side Field Station plot dropped with increasing soil depth, especially between August and October (Fig. 3). The range between the daily maximum-minimum temperatures decreased steadily with depth, and at the 30-cm depth was seldom more than one degree. In early September, due to the onset of defoliation because of Verticillium wilt and the direct exposure of the canopy probe to sunlight, recorded canopy temperatures were in excess of the air temperature at the weather station.

Colony length on roots. The distribution of colonies of both *Verticillium* species and other fungi on roots of cotton along with the apparent colony size on the media suggested each fungus occupied less than 5 mm of root length. Estimates of mean colony lengths were obtained by statistical calculations based on the number of internal and terminal colonies as described in the methods. By this method, the estimated mean colony length on roots from field plots for *V. dahliae* was 2.3 mm and for *V. tricorpus* was 1.9 mm. The mean colony length observed on roots from plants grown in the greenhouse was 2.7 and 1.8 mm, respectively, for these two fungi.

The mean lengths of colonies on roots did not vary much for either of the *Verticillium* species during the season (Table 2). The length of colonies was independent of the mean length of the root segments (16 September, Table 2) suggesting that the estimation procedure was valid. An unusually large colony length was indicated for *V. tricorpus* on the 16 September sample date. Although the reason for this is not known, each of the samples from that date yielded similar high values.

The calculated colony length of *Verticillium* spp. on roots represents mean values. Individual colony sizes were quite variable and ranged from very small to 5 mm or more. In a small portion of plated root pieces (4% of the pieces positive for *V. dahliae* and 1% for *V. tricorpus*), *Verticillium* grew out of both ends of the root piece, often with little or no *Verticillium* growing from the cortex in the middle of the piece. In the experiment where long root segments were plated, only one (out of 30 positive pieces) was positive for *V. dahliae* along its entire length (4 cm). In the root segments plated on the media, microsclerotia formed readily after 10–14 days. Colony size estimates based on length of root tissue containing microsclerotia after 14 days of incubation were about twice as long as those obtained by the preceding method. For a small portion of the observed colonies, development of microsclerotia was confined to a small area on one side of the root piece, suggesting that *Verticillium* can be restricted to very small segments of root tissue.

Distribution of colonies on roots. During the first part of the season, colonies of *V. dahliae* appeared to be randomly distributed along the root. The observed colony frequency on root pieces did not differ significantly from a Poisson distribution, as measured by the G-statistic in a goodness-of-fit test (Table 3). However, from July on, there was an indication of colony clustering since the value of the G-statistic was in excess of that expected from a Poisson distribution at the 95% confidence level (Table 3). With the exception of one date (16 Sept), there was no evidence for any deviation from a random distribution for the colonies of *V. tricorpus* (Table 3).

Vascular infection. At the end of the 1976 growing season, only *V. dahliae* could be isolated (85% frequency) from aboveground stem segments of either susceptible (SJ-2) or tolerant (SJ-5) cotton plants. Both *Verticillium* species were recovered from tomato stems: from 59% of the plants only *V. dahliae* was recovered, from 12% only *V. tricorpus* was recovered, and, from 18% of the plants, both fungi were recovered from the same stem piece.

Pathogenicity. All of the 40 isolates of *V. dahliae* obtained from localized colonies growing from cotton root segments were pathogenic on SJ-2 cotton and induced defoliation upon stem injections, although some isolates induced more rapid and more extensive defoliation than others. The 10 isolates of *V. tricorpus* were not pathogenic on cotton and, when injected into the stem, induced only a localized vascular necrosis (0.5–3 cm in length) at the injection site. Control plants injected with water exhibited no vascular necrosis.

TABLE 1. Influence of temperature on colonization of cotton roots by *Verticillium dahliae* and *V. tricorpus*[x]

Temperature (C)	Colonies per cm root[y] ($\times 10^{-2}$)	
	V. dahliae	*V. tricorpus*
31	3.8 ab	nd[z]
28	2.7 b	0.1 a
23	5.2 a	1.3 b
20	5.1 a	1.2 b

[x] Plants were grown in field soil infested with *Verticillium* in greenhouse chambers maintained at constant temperature (±2 C) as described in text.

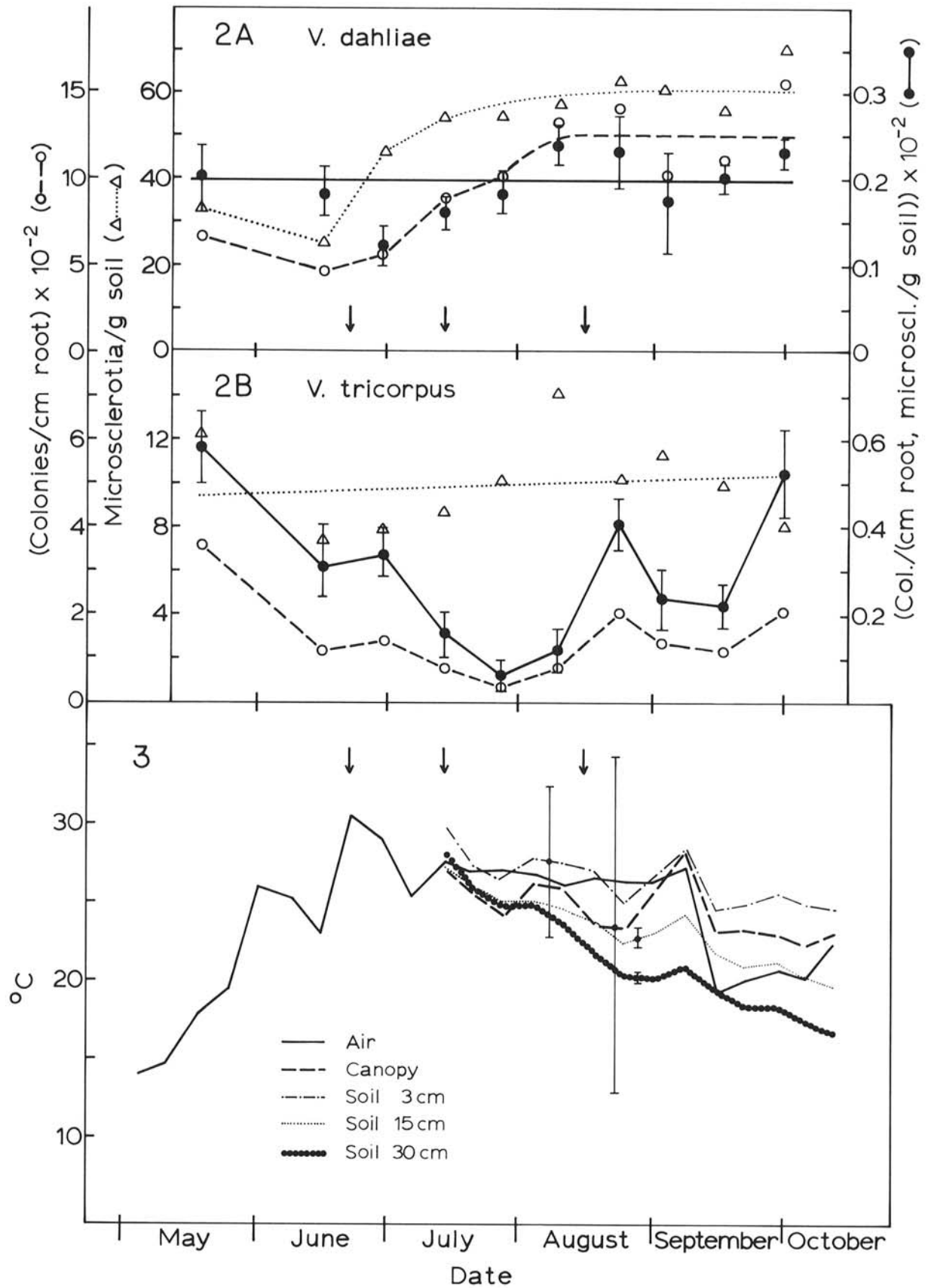
[y] Values followed by the same letter were not significantly different from each other at $P = 0.05$. LSD at 0.05 was 2.2 and 0.7 for *V. dahliae* and *V. tricorpus*, respectively.

[z] nd = none detected. Lower limit of detection was 0.1×10^{-2} colonies per centimer of root.

DISCUSSION

The findings reported here of small, apparently superficial, randomly scattered colonies of *Verticillium* on cotton and tomato roots of field plants are in agreement with the findings for greenhouse plants by Evans and Gleeson (8). In both cases, colony frequency on roots was proportional to inoculum density in soil and colonies were highly sensitive to biocides. Although Evans and Gleeson (8) used a different approach for estimating colony size on roots, the value obtained here (2.3 mm) is in good agreement with that reported by them (2 mm). This good correlation between the field studies (here), and the greenhouse studies (here and 6,8,9) indicate the results from the latter are applicable to field conditions.

Seasonal colonization of plant roots by *V. tricorpus* appears similar to colonization by *V. dahliae*. *V. tricorpus* readily colonized cotton roots forming localized colonies about 2 mm in length. Although the fungus is a pathogen of tomato (15), *V. tricorpus* has not been reported to be a pathogen of cotton. In spite of the occurrence of *V. tricorpus* on cotton roots in densities as high as were observed for *V. dahliae*, *V. tricorpus* was not detected



Figs. 2 and 3. 2, Soil inoculum densities, number of colonies on cotton roots, and colonies per unit root length per unit inoculum density for, **A,** *Verticillium dahliae* and, **B,** *V. tricorpus* as a function of time. Arrows indicate irrigations (furrow, approximately 18 cm water each time). The vertical bars represent the standard error of the fungal colonies, cm^{-1} root, microsclerotia^{-1}, g soil. **3,** Soil and air temperatures in the field plot as a function of soil depth and time. The temperatures plotted were the weekly average of the mean daily temperatures. The daily maximum-minimum range for each soil depth is indicated by the vertical bars.

in stems of wilted plants. In contrast, *V. tricorpus* was readily isolated from stems of tomato plants.

The systemic invasion of cotton plants by *V. dahliae* did not lead to systemic invasion of the fibrous root system. Roots taken from soil next to severely infected (defoliated) plants showed the same localized colonization pattern as those taken from soil next to apparently uninfected (no vascular necrosis) plants. This finding is in agreement with the reported movement of *Verticillium* in plants. Once the pathogen is in the vascular system, it moves primarily along with the transpiration stream as microconidia (4,5). Vessels are transversed by conidia and end plates by conidial germination and penetration by mycelium with subsequent microconidia formation (4,5). Such movement implies only acropedal mobility and suggests only roots lying between the point of vascular penetration and the taproot would be systemically invaded. During the examination of the many roots plated during the 1976 and 1977 seasons, a few roots (approximately 4% of colonized pieces) were encountered in which *V. dahliae* was found to grow from both ends of the root fragment. Such a pattern is suggestive of vascular colonization of the root piece but could also conceivably represent either an unusually large (1 cm or more) surface colony or two terminal colonies (as defined earlier) of two clustered localized colonies.

TABLE 2. Colony length of *Verticillium dahliae* and *V. tricorpus* on colonized cotton roots during the 1977 season

Day/month	Root piece[x] length (mm)	Colony length (mm)[y]	
		V. dahliae	*V. tricorpus*
19 May	10	2.8 (41) a	2.1 (43) bc
16 June	10	1.5 (52) a	1.8 (57) bc
30 June	10	2.0 (60) a	1.9 (49) bc
14 July	8	1.4 (29) a	1.9 (31) bc
27 July	8	1.7 (45) a	0.9 (60) c
9 August	13	3.0 (28) a	1.7 (34) bc
24 August	11	2.9 (44) a	2.2 (53) bc
2 September	11	2.5 (13) a	1.9 (30) bc
16 September	15	2.7 (10) a	5.8 (20) a
16 September	38	3.1 (36) a	6.9 (41) a
16 September	65	2.8 (30) a	7.1 (35) a
30 September	12	2.7 (29) a	3.1 (47) b
all dates		2.3 (38)	1.9 (36)[z]

[x] Mean length of the plated root pieces. Approximately 1,500 cm total root length was plated on each sampling.

[y] Values in parentheses are the coefficients of variation for estimated (see text) length of colonies on roots for each date. Values followed by the same letter were not significantly different from each other at $P = 0.05$. Standard errors ranged from 5 to 27% of reported values for individual dates and was 6% for the combined dates (after removal of variance attributable to the sampling dates) for both *V. dahliae* and *V. tricorpus*.

[z] The average colony size for *V. tricorpus* for the combined dates was 2.4 when the exceptionally high values for 16 September are included.

The roots of the four cotton cultivars that exhibited differential tolerance to Verticillium wilt (2) had similar colonization frequencies for the two *Verticillium* species. Thus, the observed differences in their rates of infection by *V. dahliae* (2) cannot be explained on this basis.

Both *V. tricorpus* and *V. dahliae* could be isolated from stems of the same tomato plant. The frequency with which this occurred came close to that expected for random distributions of the observed vascular infections. *V. dahliae* was isolated with a total frequency of 0.77 from tomato stems and *V. tricorpus* with a frequency of 0.30. If vascular infections by these two fungi were randomly distributed among the plants, the expected frequency of isolating both fungi from the same plant would be $0.77 \times 0.30 = 0.23$, the observed frequency was 0.18. Thus, systemic infection by one species does not appear to preclude systemic infection by the other.

Colonization of cotton roots by the *Verticillium* species commenced as soon as plant growth started and continued throughout the growing season. Changes in densities of *V. dahliae* on roots paralleled changes in inoculum densities in soil with a 3–4-wk lag, and the colony frequency per unit of inoculum was remarkably constant. Colonization of roots by *V. tricorpus*, in contrast, dropped off during the summer months (Fig. 2B). The constant density of soil inoculum of *V. tricorpus* indicates that either the germination or the colonization process itself was affected.

Much of the seasonal variation in root colonization by *V. tricorpus* can be explained on the basis of soil temperature effects.

TABLE 3. Frequency distribution of colonies of *Verticillium dahliae* and *V. tricorpus* on cotton root segments during 1977

Sampling date day/month	Observed (expected)[x] number of root segments with indicated number of colonies				G-statistic[y]
	0	1	2	>2	
V. dahliae					
19 May	382 (382)	34 (33)	1 (1.4)	0	0.3
16 June	518 (517)	23 (26)	2 (0.6)	0	2.2
30 June	845 (846)	59 (57)	1 (1.9)	0	0.7
14 July	1,571 (1,565)	118 (131)	12 (5.5)	0	7.4 a
27 July	991 (985)	106 (117)	10 (7)	2 (0.3)	6.8 a
9 August	948 (940)	186 (198)	22 (21)	5 (1.5)	6.2 a
24 August	459 (451)	74 (89)	14 (8.7)	2 (0.6)	7.6 a
2 September	820 (815)	103 (113)	12 (7.8)	1 (0.4)	3.6
16 September	599 (592)	108 (116)	6 (11)	7 (0.7)	22.7 a
30 September	574 (563)	115 (132)	17 (15)	6 (1.2)	12.4 a
V. tricorpus					
19 May	383 (383)	33 (32)	1 (1.4)	0	0.2
16 June	529 (529)	14 (14)	0 (0.2)	0	0.4
30 June	880 (878)	23 (26)	2 (0.4)	0	3.7
14 July	1,680 (1,679)	20 (22)	1 (0.1)	0	2.3
27 July	1,103 (1,103)	6 (6)	0 (0)	0	0.1
9 August	1,138 (1,137)	22 (24)	1 (0.2)	0	1.4
24 August	521 (520)	26 (28)	2 (0.8)	0	1.6
2 September	909 (907)	25 (28)	2 (0.4)	0	3.3
16 September	687 (684)	29 (35)	3 (0.9)	1	11.0 a
30 September	670 (670)	41 (40)	1 (1.2)	0	0.1

[x] The expected frequency distribution was calculated on the basis of a Poisson distribution where u (mean colony frequency) is total colonies/total root segments.

[y] The G-statistic was calculated on the basis of three groups having 1 df for cases with zero events in the greater than two colonies categories and on the basis of four groups having 2 df for other cases. Values followed by the letter a deviated significantly from the expected random distribution at $P = 0.05$ based on a chi-squared distribution. (Maximum values at $P = 0.05$ are 3.8 and 6.0 for 1 or 2 df, respectively).

The diurnal soil temperature variations and the changes in temperature with depth and time (Fig. 3) span the range critical to colonization of roots by *V. tricorpus* (Table 1). Thus, if soil temperature is an important factor influencing colonization, overall changes in temperature should be reflected in changes in amounts of colonization. The colonization values reported for a given sampling date necessarily represent root colonization, which occurred over a preceding time period. When allowances are made for this lag, the amount of colonization by *V. tricorpus* during the growing season is inversely related to changes in overall soil temperatures (Figs. 2B and 3). Isaac (14,15) showed that growth rates for *V. dahliae* do not decline much until temperatures in excess of 30 C are reached, while those for *V. tricorpus* start to decline around 27 C. These differences could explain, at least in part, the observed temperature sensitivity of colonization of roots by *V. tricorpus* but lack of it with *V. dahliae* (Table 1).

The random distribution of colonies on roots early in the season (through June, Table 3) is consistent with both a random distribution of inoculum of *Verticillium* in soil and with a 1:1 relationship of microsclerotia in soil and colonies on roots (i.e., a single microsclerotia does not give rise to multiple colonies on roots). Apparently inoculum was randomly distributed in the top 30 cm of soil at the beginning of the season. The various cultural operations (deep disking, bedding, etc.) probably were important in achieving this distribution. Evans and Gleeson (8) also observed random distribution of colonies of *V. dahliae* on roots in their greenhouse studies. Their results support the single microsclerotia-single colony relationship.

The observed increase in inoculum density in soil of *V. dahliae* in the spring is in agreement with earlier reports. Ashworth et al (1) observed a similar pattern of inoculum increase. The increase probably corresponds to the release of microsclerotia upon decomposition of the preceding season's infested plant residue. The increases in root colonization that followed increases in inoculum density with a 3–4 wk lag (Fig. 2A) indicate that released inoculum soon becomes functional in colonizing roots. Part of this lag may be an artifact. First, the extensive physical agitation of the soil residue during the inoculum density assay likely would affect earlier fragmentation of the decomposing infested plant residues than would occur in the soil. If this occurred, the effective inoculum would be overestimated and give suppressed values of colonies, cm^{-1} root, microsclerotia^{-1}, g soil. Secondly, root colonization values would be expected to lag soil inoculum density values, since the latter represents root colonization that occurred over a time period preceding the reported date. This also would lead to suppressed colony density values during the lag period. For these reasons, the 30 June and 14 July data for this parameter in Figure 2A were omitted in drawing the best fit line.

The increase in colony density of *V. dahliae* on roots from July on poses a question of logistics of dispersal. Without dispersal, an increase in the number of colonies per centimeter of root would not be expected. Microsclerotia released by decomposition would remain in place, resulting in pockets with exceptionally high inoculum densities because the plant debris contains many thousands of microsclerotia per gram of tissue (10). Colonization of roots by such an inoculum cluster would be seen as a single colony, possibly larger in size, in the root assay since colony centers would have to be spaced at least 3–5 mm apart to be seen as separate colonies. The data do not support the occurrence of larger colony sizes originating from clumps of inoculum before significant dispersal, since no significant differences in colony size were observed during the season (Table 2).

Limited dispersal of new inoculum would be expected to lead to a high degree of colony clustering on roots. Limited dispersal is consistent with the significant deviations from a random distribution of colonies on roots observed from mid-July onward (Table 3). The appearance of colony clustering coincided with the appearance of increased colony densities on roots (Figs. 2A and 3). Apparently, during July, sufficient dispersion of new inoculum took place to affect propagule separation in excess of 3–5 mm, the minimum distance required for the resolution of adjacent colonies on roots, but not sufficient to achieve a total random distribution in the soil.

The mechanism of dispersal is not known. The soil layer sampled for roots and inoculum density was not disturbed by cultural practices from mid-June onward and was only mildly disturbed before that (surface cultivation and furrow clearing). The large size of the microsclerotia (± 50 μm)(3) makes it unlikely that the capillary movement of water during irrigation would affect dispersal. Production of microconidia by microsclerotia (11,17), followed by their dispersal through water or other means, would also appear unlikely. Such a process should lead to a breakdown of the linear relationship between colony density on roots and density of microsclerotia (selectively measure by the soil inoculum assay) in soil. However, a consistent relationship was observed. The deviations observed (late June, early July) were opposite from that expected from a possible involvement of microconidia. Soil fauna could act as agents of dispersal (19), and their activity would be preferentially associated with decaying tissue.

In contrast to the inoculum densities for *V. dahliae*, those for *V. tricorpus* remained constant during the season (Fig. 2B). The 1977 inoculum density values for these plots also showed little or no change from the 1976 values. This lack of a measurable inoculum increase is consistent with the inability of this fungus to infect cotton systemically and probably reflects the importance of systemic infection in raising soil inoculum levels above trace levels (10,13). Tomatoes had been grown previously in the field used in this study. This combined with the use of infested tomato residues for establishing the different inoculum levels in the plots (2) probably accounts for the significant levels of *V. tricorpus* in our plots. The importance of systemic infection in inoculum buildup was further demonstrated by the much higher inoculum densities for *V. tricorpus* in the portion of the field that had been planted to tomatoes in 1975 (2) as compared with the continuous cotton part of the field used for the root colonization study.

The results reported here extend the information on colonization of roots by *V. dahliae* and show that such colonization occurs commonly in the field. Colonization of roots by *V. tricorpus* was found to be similar to that by *V. dahliae* and it could well be that many soilborne pathogens colonize roots in a similar fashion. Further studies on root colonization in relation to root growth and to systemic infection will be essential for attaining better insights on the mechanisms of colonization and the infection process.

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inoculum density assays were collected to a depth of 120 cm, at 30-cm increments, in the plots with the highest inoculum. Many tomato volunteers occurred in the portion of the field with the 1975 tomato history. To obtain comparative data on colonization of roots by the *Verticillium* species, soil samples were collected beside tomato plants permitted to grow in the alleyways of the cotton subplots.

Root sampling, 1977. During the 1977 growing season, sampling of roots started when plants were in the first true leaf stage and continued thereafter at biweekly intervals. To keep the work load manageable, only three of the five replicates of the highest inoculum density plots were sampled in this fashion. The other two replicates and some plots with lower inoculum densities were sampled sporadically during the season. Because, in the 1976 assays, no differences were observed among the cotton cultivars for colony densities (colonies, cm^{-1} root, microsclerotia^{-1}, g soil) of the *Verticillium* species on roots, the cores of soil (0–30 cm depth) from the subplots (cultivars) were bulked into a 12-core composite sample. For studies requiring long (>2-cm) root segments, samples were collected (16 September) in the plots with high inoculum density with the aid of a spade and long root pieces were retrieved upon dispersal of the soil in buckets of water.

Separation of roots from soil. Soil containing roots was usually processed within 24 hr of collection. No significant differences were found in several trials among samples processed after storage at 4 C for 12, 24, or 48 hr after collection. The soil samples were suspended in 1% sodium hexametaphosphate and stored overnight at 4 C. The macroorganic fraction was recovered by the method of Torssell et al (22). Turbulence during the flotation step was kept to a minimum to minimize root abrasion by the soil mineral fraction. Miscellaneous debris was removed by various manipulations, including selective decanting, physical removal, and extensive washings of the debris containing root pieces onto a 1-mm-mesh sieve. The final root sample was suspended in water containing 1% sodium hexametaphosphate and 0.1% Tergitol NP-10, placed on a rotary shaker, and shaken at slow speed for 10–20 min. The washing was repeated two more times with thorough rinsing of the roots with tap water on a small 125-mm-mesh sieve between washings. The final suspension was rinsed in sterile water and collected with suction onto filter paper.

Plating of roots. With the aid of fine forceps and a dissecting microscope, roots were transferred to petri plates containing media and placed sequentially about 5 mm apart in seven rows such that each plate contained about 40 linear cm of root. For the 1976 experiments, the mean length of plated root pieces was 8 mm. During the 1977 platings, root pieces less than 10 mm in length (based on visual judgment) were avoided, and the mean length of plated roots was 12 mm. Forceps were dipped into alcohol, followed by sterile water between every five to 10 transfers. Care was taken to ensure that root segments transferred were free from miscellaneous debris. After 14–21 days of incubation at 25 C, plates were examined for colonies of *Verticillium* growing from root segments based on development of microsclerotia in the media, after most aerial growth of fungi had been removed by flooding the plates with ethanol followed by gentle washing (avoiding dislocation of roots) under running tap water.

Length of plated roots was initially obtained by direct measurement. This was accomplished by placing petri plates on 1-mm grid graph paper and estimating the length of each piece to the nearest millimeter. Because root pieces did not shrink significantly during the incubation period, it was also possible to use the random line-intersect method (18,22) for estimating the length of root pieces retrieved from the plates. A plot of estimates of root lengths obtained from the same sets of roots by the direct method against those obtained by the line-intersect method yielded a plot with a slope of 0.97 and 95% confidence limits for the slope of 1.04 to 0.91. The latter method was used for obtaining the greater majority of measurements of root length.

During the 1977 season, a total of about 1,500 cm of root segments was plated for each sampling date. In the calculation of colonies per centimeter of root, the number of colonies was corrected for colony splitting due to random breakage as described below.

Analysis of cross contamination. Because forceps were not sterilized between each root transfer, a possibility of cross contamination of root pieces handled successively existed. The possible occurrence of this phenomenon was evaluated statistically. The occurrence of *V. dahliae* on adjacent root segments (placed sequentially on agar) within rows of plated segments was noted whenever it occurred. Random probability was calculated (20) using the expression derived from the binomial distribution,

$$f_i = (N/k)[(k - (i + 1)q^i - 2(k - i)q^{i+1} + (k - i - 1)q^{i+2})].$$

In this expression, f_i represents the expected frequency, assuming random distribution, for one event (root segments positive for *V. dahliae*) occurring in strings of length i separated by one or more of the other events (root segments negative for *V. dahliae*) in samples taken k at a time ($k = 5$, the average number of root segments per plated row) for a total of N events (total root segments plated), and q represents the fraction of root pieces positive for *V. dahliae*. The G-statistic was calculated on the basis of f_i (the number of f_i positive root pieces associated with each class) where

$$\Sigma(i)f_i = \Sigma(i)\hat{f}_i = Nq.$$

Estimation of colony length on roots. The mean length of colonies of the *Verticillium* species on roots of cotton was estimated by using a statistical approach. Assuming that roots break randomly during the soil sampling and crumbling steps, the frequency at which a break would occur within a colony of *Verticillium* should be proportional to the fraction of the total root length covered by such colonies, i.e.:

$$B_c / B_t = L_c / L_t \quad (1)$$

where B_c is the number of root breaks occurring within a colony of *Verticillium*, B_t is the total root breaks (total root length/mean segment length), L_c is the root length covered by colonies of *Verticillium* (total colonies × mean colony length), and L_t is the total root length. Records were kept as to whether *Verticillium* emerged from the middle of a root piece (internal colony, i.e., colonies did not appear to touch either end of the plated root piece) or whether the region of root from which the colony of *Verticillium* emerged included the end of the plated root piece (terminal colony). Colonies with questionable locations (about 10%) were divided equally between the two categories. Because breakage of a root at the site of a colony of *Verticillium* would result in two root fragments, each with a colony at one end, the total colonies of *Verticillium* split by random breaks were taken as one-half of the observed terminal colonies. The equation (derived from expression 1) used for calculation of the colony length of fungi on roots was:

$$C = R((t/2)/(i + t/2)), \quad (2)$$

where C is the mean colony length, R is the mean root segment length, t is the number of observed terminal colonies, and i is the number of observed internal colonies. Root segments positive along their entire length for *Verticillium* were not included in the internal or terminal colony counts, as is required in this statistical formulation.

Without correcting for terminal colonies, colony densities determined from root plating will overestimate the actual number of colonies, with larger errors occurring with shorter root pieces. Plating of long root segments would minimize this error. The use of a soil corer for obtaining root samples limited the mean length of plated roots to approximately 1 cm. However, this disadvantage was offset by the ability to obtain a more random root sample from plots and ease of sampling. To correct for the error introduced by colony splitting, all values for colony density on roots reported here or used in calculations were adjusted as given in expression 3,

which was derived from expression 2:

$$\text{Net colonies} = \text{Number observed colonies} \times (R/(C+R)) \quad (3).$$

In this expression, the number of observed colonies represents the total colony count without regard to the terminal or internal location of the colonies on the root segments.

To evaluate the appropriateness of the above procedures, root segments of different mean lengths were plated from the same root lots on several sampling dates (24 August and 16 September). If the estimation procedure for colony length (expression 2) is appropriate, the calculated length of colonies on roots should be independent of the mean length of the plated root segments. If the correction factor (expression 3) is appropriate, colony densities on roots should be independent of mean segment length after correction but not before correction.

Distribution of colonies on roots. To permit an evaluation of the distribution pattern of colonies along the root length, records were kept of the number of root pieces plated and the number of root pieces giving rise to two or more of each of the *Verticillium* species. The data were analyzed for deviations from a random distribution as measured by the G-statistic.

Greenhouse studies. A small-scale study of the influence of temperature on colonization of cotton roots by the two *Verticillium* species was conducted in a greenhouse in the spring of 1977. A 5-cm layer of pea-size gravel was placed in 15-L closed-bottom containers. To facilitate bottom watering, a short piece of a 5-cm-diameter plastic pipe was placed vertically along one side, and the containers were then filled with field soil from the plot with the highest inoculum density of *V. dahliae*. Cotton plants (SJ-2), one per container, were grown at 25 C (day temperature; nighttime temperature was not strictly regulated and often dropped to around 20 C) for 3 wk, at which time three plants were transferred to each of four constant temperature rooms set at 20, 23, 28, and 31 C. Soil temperatures were monitored with thermometers inserted 15 cm into the soil and were read morning and evenings at 2- to 3-day intervals. Temperatures ranged ±2 C from the mean temperature indicated. Several plants were retained in the main greenhouse (25 C). At the end of 12 wk, root samples were recovered and handled as described for the field plots.

Systemic infection and pathogenicity tests. At the end of the 1976 growing season, cotton and tomato plants were evaluated for systemic infection. Stem segments (a 2-cm segment taken 5–10 cm above the soil line) were collected from 200 plants each of the SJ-2 and SJ-5 cotton cultivars and 100 of the volunteer tomato plants. Segments were surface sterilized, and a 0.5-cm slice plated on the cellophane extract-pectate media.

Forty random isolations of *V. dahliae* and 10 of *V. tricorpus* were made from localized colonies growing from segments of cotton root plated on media. The pathogenicity of these isolates was tested on 6- to 10-wk-old cotton plants (Acala SJ-2) grown in the greenhouse. Plants were inoculated by stem injection of 5–10 μl of a conidial suspension. Conidial suspensions were obtained by washing the surface of 7- to 14-day potato-dextrose agar slants of the isolates with 5 ml of sterile water. Disease incidence was assessed by evaluation of foliar wilt symptoms and vascular necrosis after 3–4 wk.

RESULTS

Inoculum densities in soil. In the fall of 1976, densities of *V. dahliae* (0–30 cm depth) ranged from 22.0 to 18 microsclerotia per gram of soil for the plots with the most and least inoculum. Corresponding values for *V. tricorpus* were 3.4 and 0.85. Over a 4-yr period involving numerous assays, the coefficient of variation (CV) within duplicate assays from the same soil sample ranged from 17 to 31% for *V. dahliae* and from 23 to 35% for *V. tricorpus*, while the CV between samples (after removal of sums of squares attributable to assay dates, field locations, and treatments) ranged from 36 to 57% for *V. dahliae* and from 35 to 70% for *V. tricorpus*. During the 1977 season, the respective values for the CV between samples were 46 and 54%.

Inoculum densities decreased dramatically with soil depth. For *V. dahliae*, observed densities in the high inoculum plots were 32.0, 7.9, 1.8, 1.5, and 0.9 microsclerotia per gram of soil for the 0–15-, 15–30-, 30–60-, 60–90-, and 90–120-cm depths, respectively. Corresponding values for *V. tricorpus* were 5.3, 1.5, 0.58, 0.13, and 0.04 microclerotia per gram of soil. Because most of the inoculum (90%) was located in the upper 30 cm of soil, almost all root samples used in these studies were collected from this soil layer.

Growth of fungi from plated roots. The roots obtained by the isolation procedure were free from soil particles or other debris. They consisted primarily of small diameter roots (about 0.3 mm diameter). Microsclerotia or other pigmented structures were never observed in or on live roots at the time of plating. *V. dahliae* and *V. tricorpus* grew from root pieces as localized colonies similar to other fungi.

The colonies of *Verticillium*, and those of other fungi, were quite sensitive to sodium hypochlorite. A 5–15-sec exposure to 0.5% hypocholorite reduced colony numbers by half, and a 3-min exposure eliminated nearly all colonies.

There was no evidence of cross contamination of successively handled root pieces during plating. In one experiment involving the plating of 795 root segments, of which 115 were positive for *V. dahliae*, there were 10 incidences of two adjacent pieces positive for *V. dahliae* and one incidence of three adjacent positive segments. The expected frequencies on the basis of random probability were, respectively, 10.6 and 1.4. During 1976 and 1977, in 15 separate experiments for which the frequency of adjacent root pieces was evaluated, the value of the G-statistic ranged from 0.1 to 14 with only three values exceeding the value of 3.8 required to indicate significant deviation ($P = 0.05$) from the expected random distribution.

For roots derived from the same sample, colony densities obtained from short segments differed significantly from those obtained from longer segments. However, after adjustment with the correction factor (see Methods), differences were no longer significant, suggesting the factor adequately adjusted for differences due to mean root length. In one experiment (24 August sampling), uncorrected and corrected (given in parenthesis) colony densities (colonies × 100 per centimeter of root) for short (mean 5.2 mm) and longer (mean 11 mm) root segments were, respectively, 3.6 (2.9) and 4.6 (3.0) for *V. dahliae* and 3.3 (2.4) and 2.7 (2.2) for *V. tricorpus*. With longer root segments (15–65 mm, 16 September sampling), there were no significant differences within or between uncorrected and corrected colony densities, although corrected values had a slightly lower coefficient of variation (5.4 vs. 7.2%). The latter finding probably reflects the fact that, with root segments much longer than the mean colony length, the correction factor approaches one and adjustments are minor.

Relation of colony density on roots to inoculum density in soil. The number of colonies of both *V. dahliae* and *V. tricorpus* per centimeter of cotton root was directly proportional to inoculum density in the soil (Fig. 1C and D). When colonization is expressed in terms of per unit inoculum density, colony densities colonies, cm^{-1} root, microsclerotia^{-1}, g soil for *V. tricorpus* (0.014) were about eight times higher than those for *V. dahliae* (0.0017). Part of the scatter observed in these figures is probably variability due to low total number of colonies counted, since most samples represented 200–400 cm of root examined. Because both variables in Figure 1 had significant and independent error components associated with their values, Bartlett's three-group method (20) was used for evaluation of the slopes and 95% confidence limits given in the figure. For tomato, a linear relationship between colony density on roots and inoculum density in soil was also observed (Fig. 1A and B).

Colonization of roots in relation to systemic infection and cultivars. Roots of the four cotton cultivars were evaluated separately for density of colonies of *Verticillium* species during the 1976 assays. No significant differences in colony density of *V. dahliae* or *V. tricorpus* on roots were observed among the four cultivars ranging from highly susceptible (70-100) to highly tolerant (SJ-5) to Verticillium wilt. Mean colony densities on roots of the four cultivars collected from plots with both high and low

inculum densities (18.8 and 3.2 microsclerotia per gram of soil, respectively, for *V. dahliae* and 3.8 and 0.9 for *V. tricorpus*) in two separate trials were 1.9 (range 1.6–2.5, CV = 20%) $\times 10^{-1}$ for *V. dahliae* and 13.4 (range 8.1–19.8, CV 30%) $\times 10^{-3}$ for *V. tricorpus*.

The relation between occurrence of systemic infection by *V. dahliae* and root colonization was examined by plating roots from soil samples taken from within 10 cm of clusters of severely infected plants (SJ-2, defoliated) and from clusters of apparently healthy plants (SJ-2, no vascular necrosis) located within the same inoculum density plot. No significant differences were observed in colony density on roots between these two categories. Mean colony densities, ranges, and coefficients of variation were essentially the same as those given above for the cotton cultivars.

Effect of other variables on colony densities on roots. Soil depth per se had no detectable influence on the colony density of the two *Verticillium* spp. on cotton roots. Roots obtained from lower depths (to 120 cm) in the soil profile had progressively lower colony counts but these were directly related to the lower inoculum density at those depths.

All the colonization data for the 1977 season for the two *Verticillium* spp. were subjected to an analysis of variance. The coefficients of variance for colonies per centimeter of root for *V. dahliae* and *V. tricorpus*, after removal of sums of squares attributable to assay dates, field location, and soil inoculum density, were 35 and 56%, respectively.

Soil temperature had a marked effect on the colonization of cotton roots by *V. tricorpus*. In greenhouse studies, no colonization of roots was observed at 31 C, and at 28 C colonization was barely detectable (only a single colony was observed) (Table 1). The greatest amount of colonization was observed at soil temperatures of 20 and 23 C. The roots were colonized by *V. dahliae* at all the temperatures with higher values at the lower temperatures although the differences between 20–23 C and 31 C were not significant (Table 1).

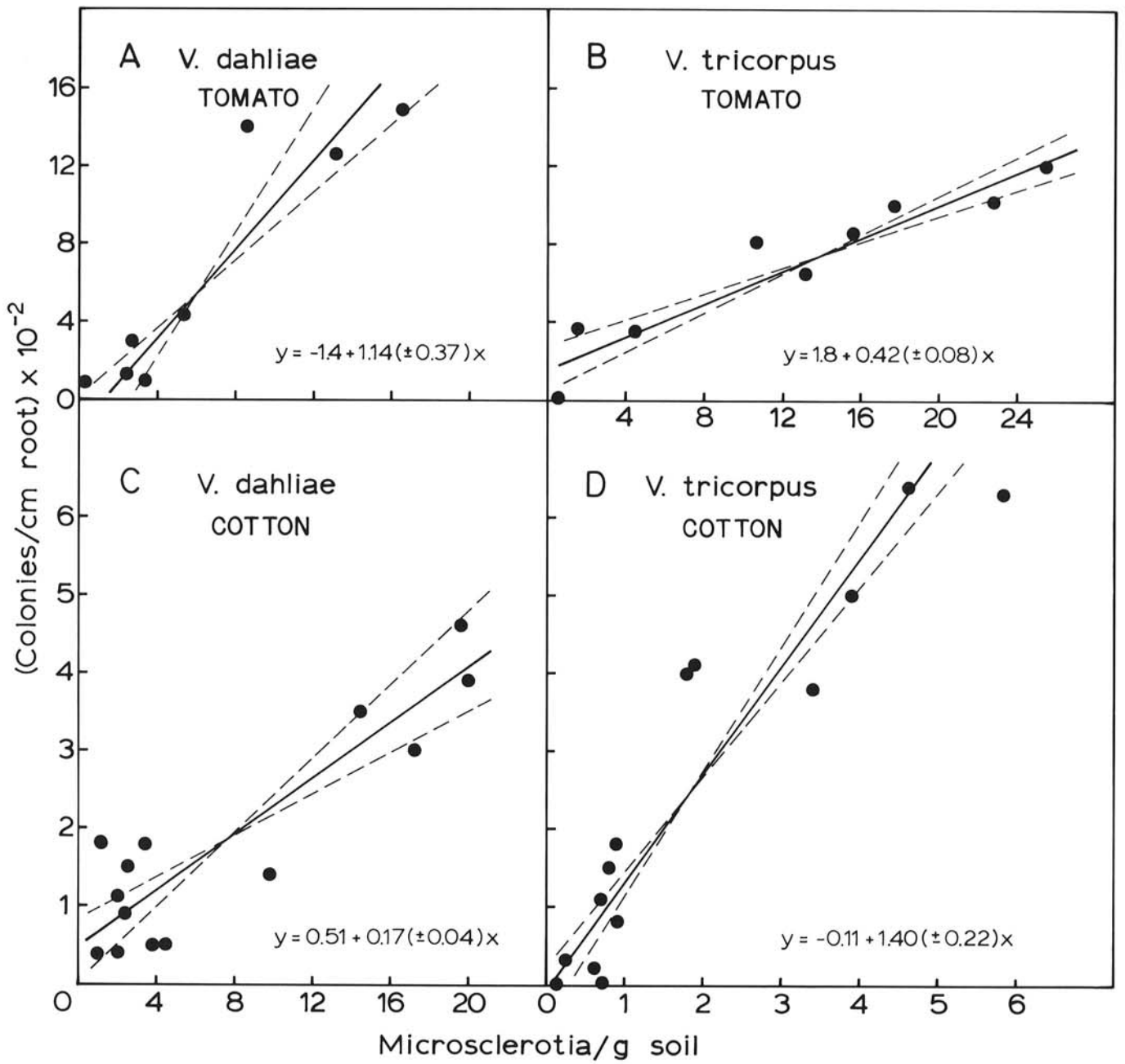


Fig. 1. Relationship between the inoculum density (microsclerotia per gram of soil) of *Verticillium dahliae* and *V. tricorpus* in soil and the observed colony density (colonies per centimeter of root) on cotton roots. Colony densities were corrected for colony splitting due to random root breakage as described in the text. The dashed lines and the value in brackets represent the 95% confidence limits of the slopes: **A,** *Verticillium dahliae* on tomato roots; **B,** *V. tricorpus* on tomato roots; **C,** *V. dahliae* on cotton roots; **D,** *V. tricorpus* on cotton roots.

Colonization of roots by the *Verticillium* species was similar for plants grown in the field and in the greenhouse. Roots retrieved from the field plot in June 1977 had 4.9 and 2.3 colonies per 100 cm for *V. dahliae* and *V. tricorpus*, respectively, whereas roots from 12-wk-old plants grown in the same soil in the 25 C greenhouse had 7.0 and 2.8 colonies per 100 cm, respectively.

Colony density on roots during the growing season. Colonization of cotton roots by *V. dahliae* had already occurred by the earliest sampling date (5 wk after planting, first fully expanded true leaf stage). *V. dahliae* exhibited a marked increase in colony density between June and August, but inoculum density also increased (from 25–70 microsclerotia per gram of soil) during this time period (Fig. 2A). Except for late June and early July, the colony density was essentially constant throughout the entire season (Fig. 2A). The lower values in late June and early July were related to the apparent lag between increases in inoculum densities in soils and corresponding increases in colony densities on roots. For this reason (see Discussion for more detailed rationale), the values for these dates were omitted in drawing a best fit regression line.

Roots of cotton were colonized by *V. tricorpus* throughout the growing season. However, the frequency of colonies on roots was not constant (Fig. 2B), with less colonization occurring during the summer months as compared with the early spring and the fall. Inoculum densities for *V. tricorpus* were essentially constant during the season (Fig. 2B). Consequently, changes in colony density paralleled the changes in colony frequency (colonies per centimeter of root) (Fig. 2B). The colony density observed toward the end of September 1977 (0.004, Fig. 2B) was only one-third that observed in October 1976 (0.014, Fig. 1D). Irrigations had no apparent effect on colonization of roots by the two *Verticillium* spp. (Fig. 2A and B).

Average soil temperatures at the West Side Field Station plot dropped with increasing soil depth, especially between August and October (Fig. 3). The range between the daily maximum-minimum temperatures decreased steadily with depth, and at the 30-cm depth was seldom more than one degree. In early September, due to the onset of defoliation because of Verticillium wilt and the direct exposure of the canopy probe to sunlight, recorded canopy temperatures were in excess of the air temperature at the weather station.

Colony length on roots. The distribution of colonies of both *Verticillium* species and other fungi on roots of cotton along with the apparent colony size on the media suggested each fungus occupied less than 5 mm of root length. Estimates of mean colony lengths were obtained by statistical calculations based on the number of internal and terminal colonies as described in the methods. By this method, the estimated mean colony length on roots from field plots for *V. dahliae* was 2.3 mm and for *V. tricorpus* was 1.9 mm. The mean colony length observed on roots from plants grown in the greenhouse was 2.7 and 1.8 mm, respectively, for these two fungi.

The mean lengths of colonies on roots did not vary much for either of the *Verticillium* species during the season (Table 2). The length of colonies was independent of the mean length of the root segments (16 September, Table 2) suggesting that the estimation procedure was valid. An unusually large colony length was indicated for *V. tricorpus* on the 16 September sample date. Although the reason for this is not known, each of the samples from that date yielded similar high values.

The calculated colony length of *Verticillium* spp. on roots represents mean values. Individual colony sizes were quite variable and ranged from very small to 5 mm or more. In a small portion of plated root pieces (4% of the pieces positive for *V. dahliae* and 1% for *V. tricorpus*), *Verticillium* grew out of both ends of the root piece, often with little or no *Verticillium* growing from the cortex in the middle of the piece. In the experiment where long root segments were plated, only one (out of 30 positive pieces) was positive for *V. dahliae* along its entire length (4 cm). In the root segments plated on the media, microsclerotia formed readily after 10–14 days. Colony size estimates based on length of root tissue containing microsclerotia after 14 days of incubation were about twice as long as those obtained by the preceding method. For a small portion of the observed colonies, development of microsclerotia was confined to a small area on one side of the root piece, suggesting that *Verticillium* can be restricted to very small segments of root tissue.

Distribution of colonies on roots. During the first part of the season, colonies of *V. dahliae* appeared to be randomly distributed along the root. The observed colony frequency on root pieces did not differ significantly from a Poisson distribution, as measured by the G-statistic in a goodness-of-fit test (Table 3). However, from July on, there was an indication of colony clustering since the value of the G-statistic was in excess of that expected from a Poisson distribution at the 95% confidence level (Table 3). With the exception of one date (16 Sept), there was no evidence for any deviation from a random distribution for the colonies of *V. tricorpus* (Table 3).

Vascular infection. At the end of the 1976 growing season, only *V. dahliae* could be isolated (85% frequency) from aboveground stem segments of either susceptible (SJ-2) or tolerant (SJ-5) cotton plants. Both *Verticillium* species were recovered from tomato stems: from 59% of the plants only *V. dahliae* was recovered, from 12% only *V. tricorpus* was recovered, and, from 18% of the plants, both fungi were recovered from the same stem piece.

Pathogenicity. All of the 40 isolates of *V. dahliae* obtained from localized colonies growing from cotton root segments were pathogenic on SJ-2 cotton and induced defoliation upon stem injections, although some isolates induced more rapid and more extensive defoliation than others. The 10 isolates of *V. tricorpus* were not pathogenic on cotton and, when injected into the stem, induced only a localized vascular necrosis (0.5–3 cm in length) at the injection site. Control plants injected with water exhibited no vascular necrosis.

TABLE 1. Influence of temperature on colonization of cotton roots by *Verticillium dahliae* and *V. tricorpus*[x]

Temperature (C)	Colonies per cm root[y] ($\times 10^{-2}$)	
	V. dahliae	*V. tricorpus*
31	3.8 ab	nd[z]
28	2.7 b	0.1 a
23	5.2 a	1.3 b
20	5.1 a	1.2 b

[x] Plants were grown in field soil infested with *Verticillium* in greenhouse chambers maintained at constant temperature (±2 C) as described in text.

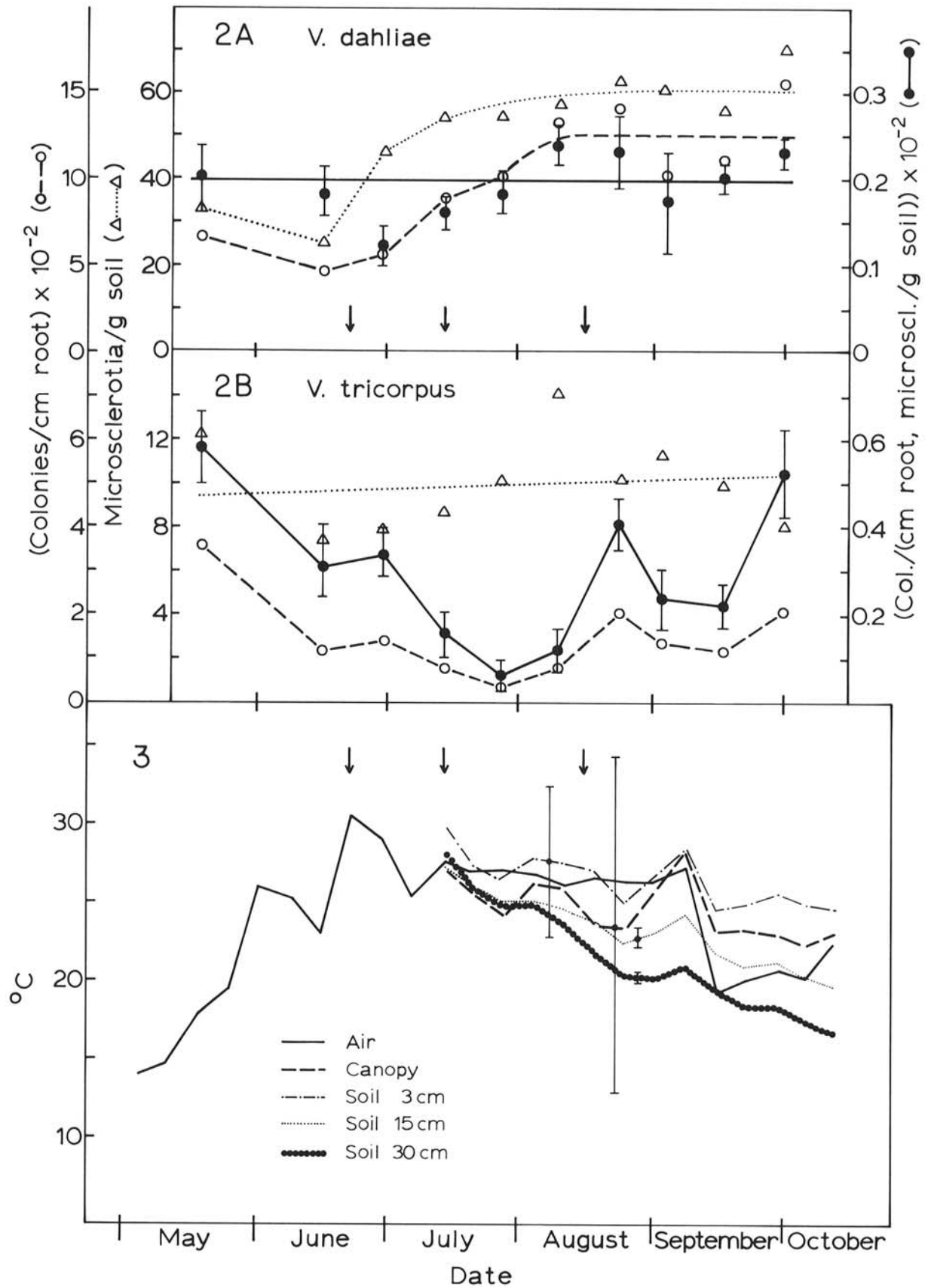
[y] Values followed by the same letter were not significantly different from each other at $P = 0.05$. LSD at 0.05 was 2.2 and 0.7 for *V. dahliae* and *V. tricorpus*, respectively.

[z] nd = none detected. Lower limit of detection was 0.1×10^{-2} colonies per centimer of root.

DISCUSSION

The findings reported here of small, apparently superficial, randomly scattered colonies of *Verticillium* on cotton and tomato roots of field plants are in agreement with the findings for greenhouse plants by Evans and Gleeson (8). In both cases, colony frequency on roots was proportional to inoculum density in soil and colonies were highly sensitive to biocides. Although Evans and Gleeson (8) used a different approach for estimating colony size on roots, the value obtained here (2.3 mm) is in good agreement with that reported by them (2 mm). This good correlation between the field studies (here), and the greenhouse studies (here and 6,8,9) indicate the results from the latter are applicable to field conditions.

Seasonal colonization of plant roots by *V. tricorpus* appears similar to colonization by *V. dahliae*. *V. tricorpus* readily colonized cotton roots forming localized colonies about 2 mm in length. Although the fungus is a pathogen of tomato (15), *V. tricorpus* has not been reported to be a pathogen of cotton. In spite of the occurrence of *V. tricorpus* on cotton roots in densities as high as were observed for *V. dahliae*, *V. tricorpus* was not detected



Figs. 2 and 3. 2, Soil inoculum densities, number of colonies on cotton roots, and colonies per unit root length per unit inoculum density for, **A,** *Verticillium dahliae* and, **B,** *V. tricorpus* as a function of time. Arrows indicate irrigations (furrow, approximately 18 cm water each time). The vertical bars represent the standard error of the fungal colonies, cm^{-1} root, microsclerotia^{-1}, g soil. **3,** Soil and air temperatures in the field plot as a function of soil depth and time. The temperatures plotted were the weekly average of the mean daily temperatures. The daily maximum-minimum range for each soil depth is indicated by the vertical bars.

in stems of wilted plants. In contrast, *V. tricorpus* was readily isolated from stems of tomato plants.

The systemic invasion of cotton plants by *V. dahliae* did not lead to systemic invasion of the fibrous root system. Roots taken from soil next to severely infected (defoliated) plants showed the same localized colonization pattern as those taken from soil next to apparently uninfected (no vascular necrosis) plants. This finding is in agreement with the reported movement of *Verticillium* in plants. Once the pathogen is in the vascular system, it moves primarily along with the transpiration stream as microconidia (4,5). Vessels are transversed by conidia and end plates by conidial germination and penetration by mycelium with subsequent microconidia formation (4,5). Such movement implies only acropedal mobility and suggests only roots lying between the point of vascular penetration and the taproot would be systemically invaded. During the examination of the many roots plated during the 1976 and 1977 seasons, a few roots (approximately 4% of colonized pieces) were encountered in which *V. dahliae* was found to grow from both ends of the root fragment. Such a pattern is suggestive of vascular colonization of the root piece but could also conceivably represent either an unusually large (1 cm or more) surface colony or two terminal colonies (as defined earlier) of two clustered localized colonies.

TABLE 2. Colony length of *Verticillium dahliae* and *V. tricorpus* on colonized cotton roots during the 1977 season

Day/month	Root piece[x] length (mm)	Colony length (mm)[y]	
		V. dahliae	*V. tricorpus*
19 May	10	2.8 (41) a	2.1 (43) bc
16 June	10	1.5 (52) a	1.8 (57) bc
30 June	10	2.0 (60) a	1.9 (49) bc
14 July	8	1.4 (29) a	1.9 (31) bc
27 July	8	1.7 (45) a	0.9 (60) c
9 August	13	3.0 (28) a	1.7 (34) bc
24 August	11	2.9 (44) a	2.2 (53) bc
2 September	11	2.5 (13) a	1.9 (30) bc
16 September	15	2.7 (10) a	5.8 (20) a
16 September	38	3.1 (36) a	6.9 (41) a
16 September	65	2.8 (30) a	7.1 (35) a
30 September	12	2.7 (29) a	3.1 (47) b
all dates		2.3 (38)	1.9 (36)[z]

[x] Mean length of the plated root pieces. Approximately 1,500 cm total root length was plated on each sampling.

[y] Values in parentheses are the coefficients of variation for estimated (see text) length of colonies on roots for each date. Values followed by the same letter were not significantly different from each other at $P = 0.05$. Standard errors ranged from 5 to 27% of reported values for individual dates and was 6% for the combined dates (after removal of variance attributable to the sampling dates) for both *V. dahliae* and *V. tricorpus*.

[z] The average colony size for *V. tricorpus* for the combined dates was 2.4 when the exceptionally high values for 16 September are included.

The roots of the four cotton cultivars that exhibited differential tolerance to Verticillium wilt (2) had similar colonization frequencies for the two *Verticillium* species. Thus, the observed differences in their rates of infection by *V. dahliae* (2) cannot be explained on this basis.

Both *V. tricorpus* and *V. dahliae* could be isolated from stems of the same tomato plant. The frequency with which this occurred came close to that expected for random distributions of the observed vascular infections. *V. dahliae* was isolated with a total frequency of 0.77 from tomato stems and *V. tricorpus* with a frequency of 0.30. If vascular infections by these two fungi were randomly distributed among the plants, the expected frequency of isolating both fungi from the same plant would be $0.77 \times 0.30 = 0.23$, the observed frequency was 0.18. Thus, systemic infection by one species does not appear to preclude systemic infection by the other.

Colonization of cotton roots by the *Verticillium* species commenced as soon as plant growth started and continued throughout the growing season. Changes in densities of *V. dahliae* on roots paralleled changes in inoculum densities in soil with a 3–4-wk lag, and the colony frequency per unit of inoculum was remarkably constant. Colonization of roots by *V. tricorpus*, in contrast, dropped off during the summer months (Fig. 2B). The constant density of soil inoculum of *V. tricorpus* indicates that either the germination or the colonization process itself was affected.

Much of the seasonal variation in root colonization by *V. tricorpus* can be explained on the basis of soil temperature effects.

TABLE 3. Frequency distribution of colonies of *Verticillium dahliae* and *V. tricorpus* on cotton root segments during 1977

Sampling date day/month	Observed (expected)[x] number of root segments with indicated number of colonies				G-statistic[y]
	0	1	2	>2	
V. dahliae					
19 May	382 (382)	34 (33)	1 (1.4)	0	0.3
16 June	518 (517)	23 (26)	2 (0.6)	0	2.2
30 June	845 (846)	59 (57)	1 (1.9)	0	0.7
14 July	1,571 (1,565)	118 (131)	12 (5.5)	0	7.4 a
27 July	991 (985)	106 (117)	10 (7)	2 (0.3)	6.8 a
9 August	948 (940)	186 (198)	22 (21)	5 (1.5)	6.2 a
24 August	459 (451)	74 (89)	14 (8.7)	2 (0.6)	7.6 a
2 September	820 (815)	103 (113)	12 (7.8)	1 (0.4)	3.6
16 September	599 (592)	108 (116)	6 (11)	7 (0.7)	22.7 a
30 September	574 (563)	115 (132)	17 (15)	6 (1.2)	12.4 a
V. tricorpus					
19 May	383 (383)	33 (32)	1 (1.4)	0	0.2
16 June	529 (529)	14 (14)	0 (0.2)	0	0.4
30 June	880 (878)	23 (26)	2 (0.4)	0	3.7
14 July	1,680 (1,679)	20 (22)	1 (0.1)	0	2.3
27 July	1,103 (1,103)	6 (6)	0 (0)	0	0.1
9 August	1,138 (1,137)	22 (24)	1 (0.2)	0	1.4
24 August	521 (520)	26 (28)	2 (0.8)	0	1.6
2 September	909 (907)	25 (28)	2 (0.4)	0	3.3
16 September	687 (684)	29 (35)	3 (0.9)	1	11.0 a
30 September	670 (670)	41 (40)	1 (1.2)	0	0.1

[x] The expected frequency distribution was calculated on the basis of a Poisson distribution where u (mean colony frequency) is total colonies/total root segments.

[y] The G-statistic was calculated on the basis of three groups having 1 df for cases with zero events in the greater than two colonies categories and on the basis of four groups having 2 df for other cases. Values followed by the letter a deviated significantly from the expected random distribution at $P = 0.05$ based on a chi-squared distribution. (Maximum values at $P = 0.05$ are 3.8 and 6.0 for 1 or 2 df, respectively).

The diurnal soil temperature variations and the changes in temperature with depth and time (Fig. 3) span the range critical to colonization of roots by *V. tricorpus* (Table 1). Thus, if soil temperature is an important factor influencing colonization, overall changes in temperature should be reflected in changes in amounts of colonization. The colonization values reported for a given sampling date necessarily represent root colonization, which occurred over a preceding time period. When allowances are made for this lag, the amount of colonization by *V. tricorpus* during the growing season is inversely related to changes in overall soil temperatures (Figs. 2B and 3). Isaac (14,15) showed that growth rates for *V. dahliae* do not decline much until temperatures in excess of 30 C are reached, while those for *V. tricorpus* start to decline around 27 C. These differences could explain, at least in part, the observed temperature sensitivity of colonization of roots by *V. tricorpus* but lack of it with *V. dahliae* (Table 1).

The random distribution of colonies on roots early in the season (through June, Table 3) is consistent with both a random distribution of inoculum of *Verticillium* in soil and with a 1:1 relationship of microsclerotia in soil and colonies on roots (i.e., a single microsclerotia does not give rise to multiple colonies on roots). Apparently inoculum was randomly distributed in the top 30 cm of soil at the beginning of the season. The various cultural operations (deep disking, bedding, etc.) probably were important in achieving this distribution. Evans and Gleeson (8) also observed random distribution of colonies of *V. dahliae* on roots in their greenhouse studies. Their results support the single microsclerotia-single colony relationship.

The observed increase in inoculum density in soil of *V. dahliae* in the spring is in agreement with earlier reports. Ashworth et al (1) observed a similar pattern of inoculum increase. The increase probably corresponds to the release of microsclerotia upon decomposition of the preceding season's infested plant residue. The increases in root colonization that followed increases in inoculum density with a 3–4 wk lag (Fig. 2A) indicate that released inoculum soon becomes functional in colonizing roots. Part of this lag may be an artifact. First, the extensive physical agitation of the soil residue during the inoculum density assay likely would affect earlier fragmentation of the decomposing infested plant residues than would occur in the soil. If this occurred, the effective inoculum would be overestimated and give suppressed values of colonies, cm^{-1} root, microsclerotia^{-1}, g soil. Secondly, root colonization values would be expected to lag soil inoculum density values, since the latter represents root colonization that occurred over a time period preceding the reported date. This also would lead to suppressed colony density values during the lag period. For these reasons, the 30 June and 14 July data for this parameter in Figure 2A were omitted in drawing the best fit line.

The increase in colony density of *V. dahliae* on roots from July on poses a question of logistics of dispersal. Without dispersal, an increase in the number of colonies per centimeter of root would not be expected. Microsclerotia released by decomposition would remain in place, resulting in pockets with exceptionally high inoculum densities because the plant debris contains many thousands of microsclerotia per gram of tissue (10). Colonization of roots by such an inoculum cluster would be seen as a single colony, possibly larger in size, in the root assay since colony centers would have to be spaced at least 3–5 mm apart to be seen as separate colonies. The data do not support the occurrence of larger colony sizes originating from clumps of inoculum before significant dispersal, since no significant differences in colony size were observed during the season (Table 2).

Limited dispersal of new inoculum would be expected to lead to a high degree of colony clustering on roots. Limited dispersal is consistent with the significant deviations from a random distribution of colonies on roots observed from mid-July onward (Table 3). The appearance of colony clustering coincided with the appearance of increased colony densities on roots (Figs. 2A and 3). Apparently, during July, sufficient dispersion of new inoculum took place to affect propagule separation in excess of 3–5 mm, the minimum distance required for the resolution of adjacent colonies on roots, but not sufficient to achieve a total random distribution in the soil.

The mechanism of dispersal is not known. The soil layer sampled for roots and inoculum density was not disturbed by cultural practices from mid-June onward and was only mildly disturbed before that (surface cultivation and furrow clearing). The large size of the microsclerotia (± 50 μm)(3) makes it unlikely that the capillary movement of water during irrigation would affect dispersal. Production of microconidia by microsclerotia (11,17), followed by their dispersal through water or other means, would also appear unlikely. Such a process should lead to a breakdown of the linear relationship between colony density on roots and density of microsclerotia (selectively measure by the soil inoculum assay) in soil. However, a consistent relationship was observed. The deviations observed (late June, early July) were opposite from that expected from a possible involvement of microconidia. Soil fauna could act as agents of dispersal (19), and their activity would be preferentially associated with decaying tissue.

In contrast to the inoculum densities for *V. dahliae*, those for *V. tricorpus* remained constant during the season (Fig. 2B). The 1977 inoculum density values for these plots also showed little or no change from the 1976 values. This lack of a measurable inoculum increase is consistent with the inability of this fungus to infect cotton systemically and probably reflects the importance of systemic infection in raising soil inoculum levels above trace levels (10,13). Tomatoes had been grown previously in the field used in this study. This combined with the use of infested tomato residues for establishing the different inoculum levels in the plots (2) probably accounts for the significant levels of *V. tricorpus* in our plots. The importance of systemic infection in inoculum buildup was further demonstrated by the much higher inoculum densities for *V. tricorpus* in the portion of the field that had been planted to tomatoes in 1975 (2) as compared with the continuous cotton part of the field used for the root colonization study.

The results reported here extend the information on colonization of roots by *V. dahliae* and show that such colonization occurs commonly in the field. Colonization of roots by *V. tricorpus* was found to be similar to that by *V. dahliae* and it could well be that many soilborne pathogens colonize roots in a similar fashion. Further studies on root colonization in relation to root growth and to systemic infection will be essential for attaining better insights on the mechanisms of colonization and the infection process.

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