Infection and Colonization of Potato Roots by *Verticillium dahliae* as Affected by *Pratylenchus penetrans* and *P. crenatus*

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Salaries and research support provided by the United States-Israel Binational Agricultural Research and Development Fund (BARD) and state and federal funds appropriated to the Ohio Agricultural Research and Development Center, Ohio State University. Manuscript 194-95.

We thank S. A. Miller and D. Fulton for assistance with the production and testing of the antiserum and J. Carson and W. Beery for excellent technical assistance.

Accepted for publication 18 March 1996.

ABSTRACT

Bowers, J. H., Nameth, S. T., Riedel, R. M., and Rowe, R. C. 1996. Infection and colonization of potato roots by *Verticillium dahliae* as affected by *Pratylenchus penetrans* and *P. crenatus*. Phytopathology 86:614-621.

Factorial greenhouse experiments were conducted to test hypotheses concerning the nature of the interaction between *Verticillium dahliae* and *Pratylenchus penetrans* or *Pratylenchus crenatus* in potato early dying. Soil was infested with known densities of *V. dahliae* and *P. penetrans* or *V. dahliae* and *P. crenatus*, and plants were destructively harvested 3, 5, 7, and 9 weeks after planting. Root samples were excised using a grid method and stained using an immunoenzymatic procedure. Root infection by *V. dahliae* was through the root tip and the zone of elongation, regardless of the presence of nematodes. Discrete colonies in the cortex were not observed, but rather the hyphae progressed into the cortex and grew towards the developing vascular tissues. Hyphae penetrated and grew in the young xylem elements and then colonized the vascular cylinder, or they were prevented from further development by a visible host response. Five weeks after planting, roots grown in soil infested with *V. dahliae* alone had a very low percentage of infected root tips (1.2%), which was significantly less ($P < 0.02$) than infected roots growing in soil infested with either *V. dahliae* and *P. penetrans* or *V. dahliae* and *P. crenatus* (2.3 and 2.5%, respectively). Roots were colonized by *V. dahliae* to a significantly greater extent ($P < 0.01$) when grown in soil infested with *V. dahliae* and *P. penetrans* (0.13 cm of colonized root/m of root) than in soil infested with *V. dahliae* alone or with *V. dahliae* and *P. crenatus* (0.05 and 0.02 cm of colonized root/m of root, respectively). This trend continued with differences among treatments more pronounced after 7 weeks. Low levels of initial root infection and colonization resulted in high incidences of stem colonization by *V. dahliae* in treatments with *V. dahliae* and *P. penetrans* (58% after 5 weeks and 100% after 7 weeks). Infection by *V. dahliae* was not observed to be associated with the site of nematode feeding, and the effect of nematodes on initial infection may not be species-specific. The interaction between *V. dahliae* and *P. penetrans* in potato early dying may occur within the root early in the infection process, resulting in an altered or delayed host response to colonization by *V. dahliae*.

Premature vine death and declining yields are often a problem where potatoes (*Solanum tuberosum* L.) have been in production for many years (37,41). This syndrome, called potato early dying (PED), is a factor limiting potato production in many areas of the United States (6,26,29,30,37,42). The disease is associated with the soilborne fungus *Verticillium dahliae* Kleb., but can occur as a disease complex that includes *V. dahliae* and the root-lesion nematode *Pratylenchus penetrans* (Cobb) Filipjev & Schuurmans Stekhoven (37,41). Studies in Ohio (30,39,42), Wisconsin (26,29), and Israel (27) have shown that *V. dahliae* and root-lesion nematodes can interact synergistically; together they cause severe symptom development (vascular wilt) and significant yield reductions at population levels that have little or no effect when each species is present individually. The involvement of *P. penetrans* in PED is important because of its ability to activate relatively low populations of *V. dahliae* that would otherwise not be biologically significant in causing disease. In contrast, *P. crenatus* Loof also is present in potato soils in Ohio (3,46) and has been found not to interact with *V. dahliae* in PED (39).

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Publication no. P-1996-0417-01R

Root injury caused by nematode feeding has been considered a likely mechanism for the synergism of *V. dahliae* and *P. penetrans* in PED. Wounded roots may either stimulate the germination of dormant microsclerotia by increased root exudation or facilitate access for the fungus to the vascular cylinder; these also may occur together. *P. penetrans* and *P. crenatus* both have similar feeding habits (25,32) and reproduce well on potato roots (39), but only *P. penetrans* has been shown to interact with *V. dahliae* in this disease syndrome (39,42). The species-specific nature of this interaction in PED suggests that the mechanism(s) may be more complex. Before the mechanism of the interaction can be determined, it is first necessary to ascertain where the interaction occurs in the sequence of pathogenesis. Our objectives were to observe the infection and colonization of potato roots by *V. dahliae* and to focus studies on those aspects of the infection and colonization process in which the presence of associated nematode feeding may influence fungal activity.

Two hypotheses were tested in our attempt to elucidate the ecology of the interaction. First, since initial infection by *V. dahliae* is believed to occur in the area of the root tip (13,36), the interaction may occur in the rhizosphere or at the root surface. In this case, the two *Pratylenchus* spp. may alter the physiology of the host in different ways so as to change the composition of root exudates, which may in turn act differentially on microsclerotia of

V. dahliae and influence rhizosphere width (5,40,44). This would lead to an increase in the number of contacts between roots and microsclerotia and, thus, the number of root tips infected by *V. dahliae* may be greater with root systems infected with *P. penetrans*. Second, if root injury as a result of nematode feeding merely removes physical barriers for the fungal hyphae and does not promote a physiological response from the host, then *V. dahliae* would be expected to infect and colonize potato roots to the same degree regardless of whether *P. penetrans* or *P. crenatus* also is present, but to a greater degree than the presence of *V. dahliae* alone.

MATERIALS AND METHODS

Factorial experiments were conducted in a greenhouse in 13.0-cm-diameter pots containing approximately 1,000 cm^3 of a well-decomposed, organic muck soil (Rifle peat) (12). Soil was fumigated in the field prior to collection, brought to the greenhouse, and stored in large plastic cans until use. Treatments consisted of soil infested with *V. dahliae* at 0 and 50 microsclerotia per cubic centimeter and nematodes (*P. penetrans* or *P. crenatus*) at 25 vermiforms per 100 cm^3 of soil. Each test contained six replicate pots per *Verticillium-Pratylenchus* treatment per sample date. A single isolate of *V. dahliae* (P-7, vegetative compatibility group 4A) was used throughout the experiments (2,23,24). Microsclerotial inoculum of *V. dahliae* was prepared as described previously (12). Vermiforms of *P. penetrans* and *P. crenatus* from Ohio potato soils were produced in monoxenic tissue culture and prepared as described previously (31,38,42). Single-eye potato seedpieces (cv. Superior) obtained from *Verticillium*-free stock were used as planting material (42). Plants were harvested at 3, 5, 7, and 9 weeks after planting.

Entire root systems from each plant were harvested at each sample date. Roots were carefully washed free of soil under a gentle stream of water in a large bucket, and samples were excised using a modified grid method (18). The entire root system was placed on a glass plate in a shallow tray of water, and the roots were teased apart with fine dissecting needles until they uniformly covered a specific area of the glass plate in a single layer. The glass plate with roots was removed carefully from the tray so as not to disturb the roots and then placed over a grid of 1-cm squares. The grid was marked with several (the number depending on grid size) randomly arranged 4-cm^2 areas (2 × 2 cm) marked in a single color. The total area of one color corresponded to 10% of the total grid area. Five to eight 10% sampling areas (identified by different colors) were arranged on one grid, enabling one to collect five to eight 10% root samples without disturbing or redistributing the roots. This insured a random sampling of roots without regard to root age, class, or order. Typically, three to four 10% root samples per plant (depending on root density) were excised by carefully cutting the roots with a scalpel and removing those roots lying over areas of one color on the grid. Harvested root samples were placed immediately in a fixative solution consisting of 1.0% formaldehyde and 2.0% glutaraldehyde in 0.025 M sodium cacodylate buffer (pH 7.2) until stained for the presence of *V. dahliae* using an immunoenzymatic staining technique (17).

In preliminary studies, colonization of roots by *V. dahliae* was assessed by plating root samples on or embedding them in a semiselective medium (sodium polygalacturonic acid medium [30]) and observing colony growth of *V. dahliae* from the roots (9,19,28). Roots were surface-sterilized for 3 min in 0.5% NaOCl, rinsed twice in sterile distilled water, and blotted dry on sterile paper towels. Roots were either distributed over the top of solidified medium or placed in molten medium. Petri plates with molten medium were placed on a slide warming tray set at 48 to 50°C to keep the medium from solidifying while the roots were teased apart and evenly distributed. In the studies reported here, however, infection and cortical and vascular colonization of the roots were assessed using an immunoenzymatic staining technique to visualize colonies of *V. dahliae* both in the cortex and in the vascular system (16,17). This serological procedure is capable of locating hyphae of specific fungi and permits direct, selective observation within root tissues. The immunoenzymatic staining technique is based on polyclonal antibodies prepared against a soluble protein extract of *V. dahliae* (isolate P-7).

The protein extract, antibody serum, and root-staining protocol were accomplished according to the methods of Gerik et al. (17). Soluble proteins for use as an antigen were extracted from cultures of *V. dahliae* (isolate P-7) growing in Czapek-Dox broth (Difco Laboratories, Detroit) amended with 0.5 g of yeast extract per liter (17). Protein content of the resulting suspension was determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA). One milliliter of the soluble protein suspension was dialyzed against three changes of phosphate-buffered saline, and an emulsion was prepared by adding 1.0 ml of Freund's incomplete adjuvant. Two white, female New Zealand rabbits were each injected intramuscularly with 1.0 ml of emulsion containing 0.5 mg of protein/ml. Booster injections were given 10 and 108 days after initial injections. Rabbits were bled from a marginal vein in the left ear 36, 57, 119, and terminally 136 days after the initial inoculation. The serum was separated by centrifugation, and 0.01% sodium azide was added before storage at –10°C.

Antibody titre and specificity were determined using indirect enzyme-linked immunosorbent assays (ELISA) (4). Serum specificity from the first and second bleeds (36 and 57 days after inoculation, respectively) was checked against the following fungi commonly isolated from potato roots grown in the organic muck soil: *Dorotomyces* sp. (MR12), *Penicillium* sp. (MR14), *Aspergillus* sp. (MR19), *Fusarium* sp. (MR30), and *Trichoderma* sp. (MR32). Healthy potato roots, *V. dahliae* isolate P-7, and the protein antigen (Ag) were included as controls. The test fungi were grown in Czapek-Dox broth (Difco Laboratories) medium, harvested, and ground in liquid nitrogen. The resulting powder was reconstituted in a microplate sensitizing solution (0.05 M carbonate/bicarbonate buffer, pH 9.6) and diluted 1:100 before coating microtitre plates. Antibody dilutions of 1:100 through 1:1,000 were used in the ELISA. Serum specificity from the final bleed (136 days after inoculation) was assayed against *V. dahliae* isolates P-7 and P-105, *Colletotrichum acutatum* (Ca-1), *Colletotrichum* spp. (isolates 241 and 342), *Fusarium solani* (isolate 221), *F. oxysporum* (isolate 500), *F. culmorum* (isolate 347), *Penicillium* sp. (VSI-12), *Trichoderma hamatum* (isolate 459), and *Aspergillus* sp. (MR16). Fungi were grown and harvested as above and then lyophilized. Protein content of the lyophilized mycelium was determined using the Bio-Rad Protein Assay (microassay procedure, Bio-Rad Laboratories). Lyophilized mycelium were suspended in the sensitizing solution and added to microtitre plates at 5.0 µg of protein/ml. An antibody dilution of 1:800 was used in the ELISA for specificity. For the actual staining procedure, an antibody dilution of 1:500 was used.

After staining, each root sample was suspended in a 1:1 (vol/vol) solution of glycerol and 0.025 M sodium cacodylate buffer (pH 7.2) and redistributed in several glass petri dishes. Total root length was estimated using the line intersect method (45), and the length of root colonized by *V. dahliae* (that portion of the root that contained a detectable presence of the pathogen internal to the root) was estimated by using an eyepiece micrometer in a stereoscope. The data were expressed as the amount of root colonized per unit length of root. At the same time, uninfected and infected root tips were counted. A root tip was scored as infected when stained hyphae were observed within internal tissues. The data were expressed as the percent infected root tips and the number of root tips per unit length of root. One 10% root sample per plant was stained with acid-fuchsin (7) to enumerate

nematode populations in the roots to determine if nematode numbers within tissues were correlated with, or spatially related to, the extent of colonization by *V. dahliae*. These data also were used to determine if *P. penetrans* and *P. crenatus* infect potato roots equally. Vascular colonization of the stem over time (using the same test plants) was assessed by plating excised basal stem segments (0 to 2 cm above the soil line) on water agar medium and recording the number of plants at each sample date in which *V. dahliae* grew out of the vascular bundles. The observations and data presented below are the results of two trials of the experiment. Single degree-of-freedom contrasts were used to determine differences among treatments at each sample time.

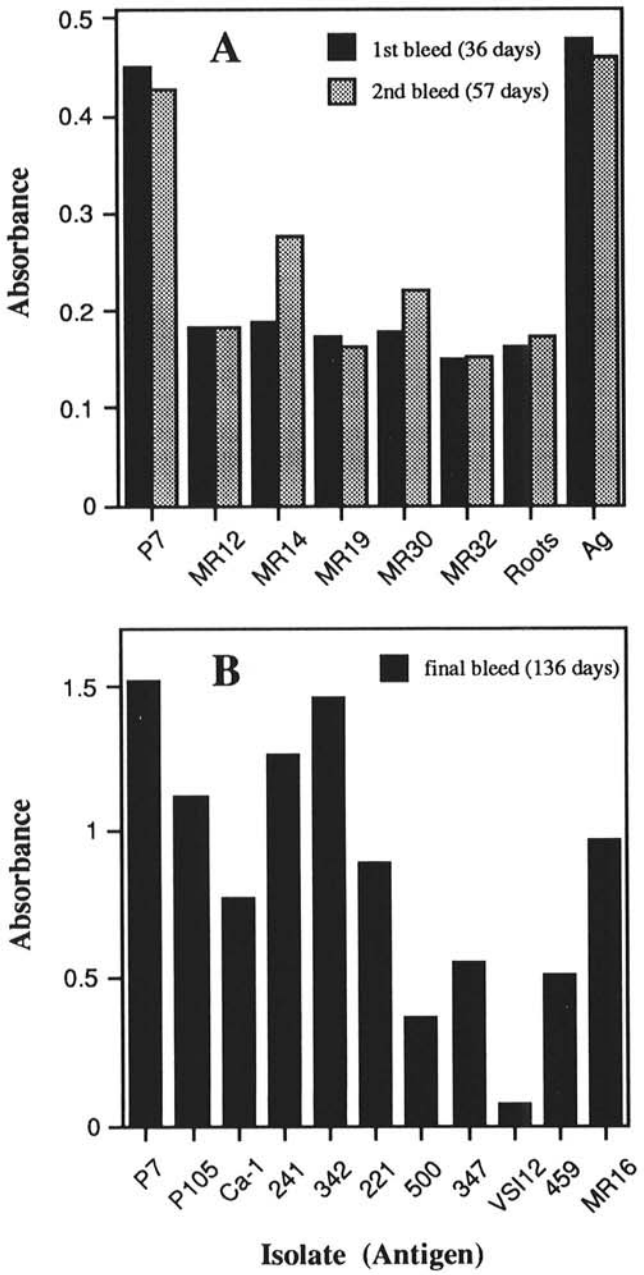

Fig. 1. ELISA antibody specificity tests for antisera collected **A,** 36 and 57 days after immunization (antibody dilution of 1/100) and **B,** 136 days after immunization (antibody dilution of 1/800). The immunogen (Ag) was a soluble protein extract from *Verticillium dahliae* isolate P-7. Fungi tested were **A,** *Dorotomyces* sp. (MR12), *Penicillium* sp. (MR14), *Aspergillus* sp. (MR19), *Fusarium* sp. (MR30), *Trichoderma* sp. (MR32), and healthy potato roots; and **B,** *V. dahliae* (isolate P-105), *Colletotrichum acutatum* (Ca-1), *Colletotrichum* spp. (isolates 241 and 342), *Fusarium solani* (isolate 221), *F. oxysporum* (isolate 500), *F. culmorum* (isolate 347), *Penicillium* sp. (VSI-12), *Trichoderma hamatum* (isolate 459), and *Aspergillus* sp. (MR16).

RESULTS

When root samples were embedded in the semiselective agar medium, *V. dahliae* was never observed growing from roots, even from those sampled after 5 weeks of plant growth. Additionally, the medium generally became colonized by a large number of other fungi that may have inhibited growth of *V. dahliae*. Embedding the roots in the medium also may have inhibited growth because of oxygen deprivation. Surface-sterilizing the roots in NaOCl reduced the number of contaminating fungi, but did not improve recovery of *V. dahliae*. *V. dahliae* was recovered from roots placed on the agar surface but, once again, contaminating fungi were numerous. Plating roots on agar was deemed an unreliable and inefficient assay method and was abandoned.

A polyclonal antiserum was produced against a soluble protein preparation of *V. dahliae*. In specificity tests of antiserum collected 36 and 57 days after injection of the protein antigen, the polyclonal antibody was found to be specific for *V. dahliae* P-7 with approximately a twofold increase in absorbance readings compared with the test fungi or potato roots (Fig. 1A). Antiserum collected 119 days after injection was not tested for specificity, but used only to assess titre. The antiserum from the final bleed was not as specific as was the antiserum from the earlier bleeds (Fig. 1B). The antiserum cross-reacted with isolates of *Colletotrichum* (isolates 241 and 342, probably *C. coccodes*) and *F. solani* (isolate 221) and, relative to some fungi, reacted less to *V. dahliae* isolate P-105. Cross-reaction with *T. hamatum* (isolate 459) and *Aspergillus* sp. (isolate MR16) was not considered a problem because these fungi are usually found on root surfaces rather than internally. Cross-absorption of the antibodies in antiserum from the final bleed with *Colletotrichum* sp. (isolate 342) significantly reduced the cross-reactivity of the antibodies, but the titre was reduced to a level that was impractical for use in the root-staining procedure.

Since the other fungi included in these tests were believed to be found primarily on the root surface and *V. dahliae* internally, the specificity of the antiserum was considered adequate for use in the serological-staining procedure with roots grown in fumigated soil. In preliminary experiments, internal hyphae that were stained morphologically resembled *V. dahliae* growing on agar medium (Fig. 2A, B, C). Some hyphae loosely attached to the root and hyphal mats on the root surface also were stained, but they did not morphologically resemble hyphae of *V. dahliae*. In a visual assessment, the lack of complete specificity was not considered a problem since decisions could be made on a root-to-root basis (e.g., internal versus external hyphae). In the experiments reported here, only the length of roots with stained internal hyphae were evaluated. Antisera from the first two collection times (36 and 57 days after injection) were used the most in the staining procedure; however, antiserum from the final bleed was used when the supply of antisera from the previous bleeds was exhausted. Antiserum from the bleed 119 days after injection was not used in any experiment.

Experimental trials with other batches of soil in which the antiserum collected after 136 days was used were not successful because of excessive, nonspecific staining in the control (uninfested) treatments. In these experiments, the presence of *V. dahliae* in basal stem segments was not detected, even in plants grown in soil infested with *V. dahliae*. In contrast, nonspecific staining in uninfested treatments in experiments reported here using the first batch of soil and antisera from three different bleeds was low compared with *V. dahliae*-infested treatments (Fig. 3E). *V. dahliae* was detected in basal stem segments of plants grown in infested soil (Fig. 3F), but not from plants grown in uninfested soil.

Several qualitative assessments of root infection and colonization by *V. dahliae* using the immunoenzymatic staining technique were made based on observations of 1,493 m of root and 33,393 root tips in treatments with and without nematodes (Fig. 2). In-

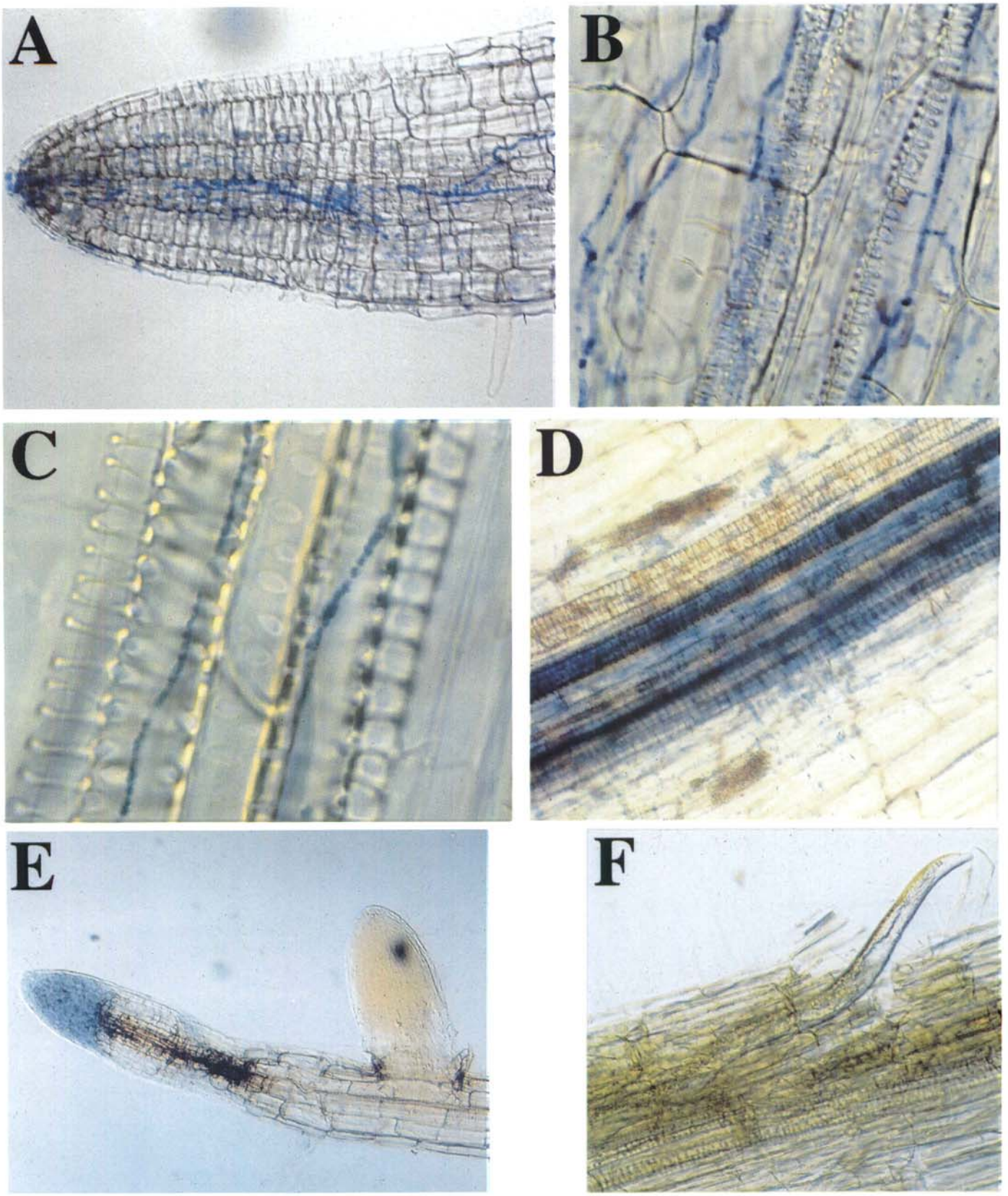

Fig. 2. Infection and colonization of potato roots by *Verticillium dahliae* (**A** to **E**) and *Pratylenchus penetrans* (**F**). All roots were stained using an immunoenzymatic staining technique to locate hyphae of *V. dahliae* within root tissues (16). Hyphae of *V. dahliae* stained blue and, in all photomicrographs, are located internally (not on the root surface). **A,** Infection of potato root tip by *V. dahliae* with hyphae growing internally towards the developing vascular cylinder (×160). **B,** Hyphae of *V. dahliae* growing in the cortex near the endodermis of young xylem elements just behind the root tip (×400). **C,** Segment of a young vascular bundle showing hyphae of *V. dahliae* growing through the lumens of tracheae in the area just behind the root tip (×1,000). **D,** Xylem elements heavily colonized by *V. dahliae* (×400). **E,** Infected root tip showing the typical host response (brown-colored cells in vascular cylinder) limiting further colonization by *V. dahliae*, and a uninfected emerging lateral root tip (×63). **F,** Root-lesion nematode *P. penetrans* feeding ı a potato root with associated cortical cell necrosis and no stained hyphae of *V. dahliae* (×160).

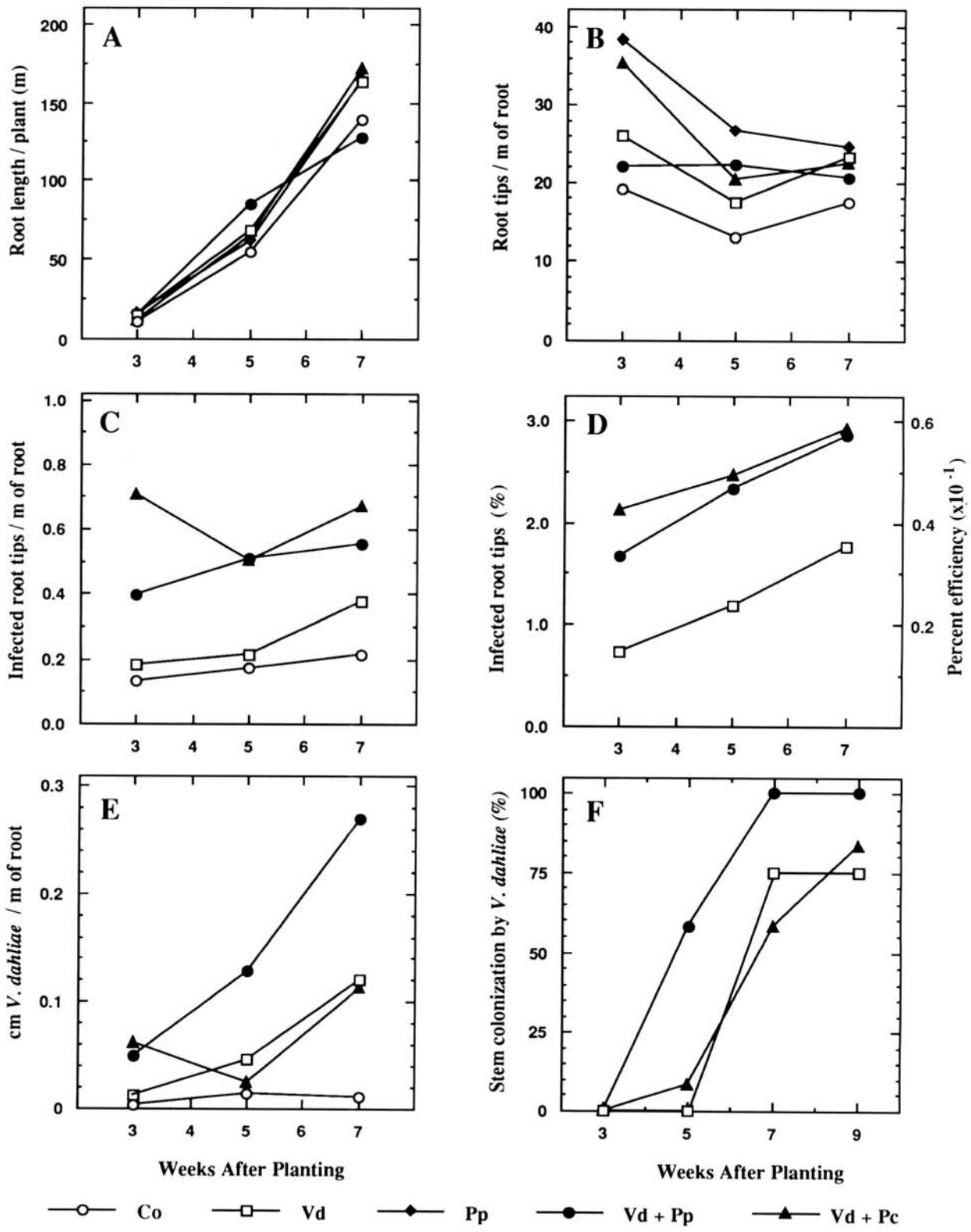

Fig. 3. Effect of *Verticillium dahliae* (Vd) and *Pratylenchus penetrans* (Pp) or *P. crenatus* (Pc), alone or in combination, and the uninfested control (Co) over time on **A,** total potato root length; **B,** number of root tips per meter of root; **C,** number of infected root tips per meter of root; **D,** percent infected root tips (left axis) and percent inoculum efficiency (right axis); **E,** centimeters of root containing a detectable presence of *V. dahliae* per meter of root (= percent root colonized); and **F,** percent stems colonized by *V. dahliae*. Each point represents the average of 12 observations (one 10% root sample [**A** to **E**] or one stem [**F**] per plant, six replicate plants per treatment for each of two trials).

fection of roots by *V. dahliae* was almost entirely through the root tip or the area just behind the root tip in the zone of elongation (Fig. 2A). Discrete colonies of *V. dahliae* in the cortex were not observed along the length of infected roots. Following infection, the hyphae progressed into the cortex and grew towards the developing vascular tissues (Fig. 2B). Hyphae of *V. dahliae* penetrated and grew in the young xylem elements (Fig. 2C) and then colonized the vascular cylinder (Fig. 2D), or they were prevented from further development by a visible host response (Fig. 2E). The host response consisted of dark brown cells (the staining technique may intensify or alter the color or consistency of the host response) almost always confined to the vascular cylinder. This reaction was in almost all infected root tips and some that did not contain visible hyphae of *V. dahliae*. Hyphae of *V. dahliae* were never observed beyond the area of the host response and, in some instances, an uninfected lateral root emerged after *V. dahliae* colonized the main root tip and meristematic activity was lost (Fig. 2E). In some root tips, this host response was formed further back along the root and *V. dahliae* was observed in the young vascular cylinder up to the point of the host response. No differences in the host response to infection by *V. dahliae* were observed in roots with *P. penetrans* or *P. crenatus*. Hyphae of *V. dahliae* were not associated spatially with nematode feeding or necrosis of cortical cells that resulted from nematode feeding (Fig. 2F). The host response (Fig. 2E) could be distinguished from nematode injury that was confined to the cortical cells (Fig. 2F).

The data presented below are the pooled average of two trials of the experiment. Root tip infection and vascular colonization were observed 3 weeks after planting. Above-ground stem colonization was first detected 5 weeks after planting, and visible symptom development was first evident 8 to 9 weeks after planting. The average rate of root growth per plant in the two trials generally was linear, with total root length increasing from approximately 10 to 15 m after 3 weeks to 54 to 85 m after 5 weeks and 127 to 172 m after 7 weeks for all treatments (Fig. 3A). There was a decrease in the average rate of root growth for plants grown in soil infested with *V. dahliae* and *P. penetrans* between 5 and 7 weeks after planting compared with the other treatments. After 7 weeks, the average root length per plant for roots grown in soil infested with *V. dahliae* and *P. penetrans* (127.4 m) was significantly less than for those grown in soil infested with *V. dahliae* alone (163.7 m, $P = 0.06$) or *V. dahliae* and *P. crenatus* (171.2 m, $P = 0.03$). The average number of root tips per meter of root varied widely 3 weeks after planting, but approached a constant after 5 and 7 weeks, averaging about 20 root tips/m of root for all treatments (Fig. 3B). There were no significant differences ($P = 0.20$) in the average number of root tips among treatments 7 weeks after planting. Potato root systems were extremely fibrous, with the vast majority of roots being laterals averaging only 0.23 mm in diameter. There were approximately 10 larger main roots originating from the basal portion of the stem of each plant that were 0.5 to 1.0 mm in diameter. Larger roots were capable of growing, on the average, 1.5 cm/day.

The number of infected root tips per meter of root increased slightly from 3 to 7 weeks after planting in all treatments (Fig. 3C). After 5 weeks, the number of infected root tips was significantly greater ($P = 0.02$) for roots grown in soil infested with *V. dahliae* and either nematode species (0.51 and 0.50 infected root tips/m of root for *V. dahliae* plus *P. penetrans* and *V. dahliae* plus *P. crenatus*, respectively) than roots grown in soil infested with *V. dahliae* alone (0.21 infected root tips/m of root). The correspondence between the near constant production of root tips per meter of root and the linear increase in root length over time suggested that root tips became infected at approximately a constant rate. The linear increase in root tip infection is clearly seen when the data are plotted as the percentage of infected root tips over time (Fig. 3D, left axis). On average, the percentage of infected root tips was low with a range of 0.7 to 3.0% for all treatments and, as above, the percentage of infected root tips was significantly greater ($P = 0.02$) for roots grown in soil infested with *V. dahliae* and either nematode species (*V. dahliae* plus *P. penetrans* and *V. dahliae* plus *P. crenatus*) than for roots grown in soil infested with *V. dahliae* alone 5 weeks after planting. The efficiency of the inoculum for infection was defined as that portion of propagules on or adjacent to the infection court that induces successful infection of the root (8), and calculated as the multiple infection transformation of the proportion of infected root tips per cubic centimeter divided by the inoculum density of microsclerotia in the soil. Inoculum efficiency was low and, on average, ranged from 0.014 to 0.060% for all treatments (Fig. 3D, right axis). Plants grown in soil infested with *V. dahliae* and *P. penetrans* or *V. dahliae* and *P. crenatus* had a higher average number of infected root tips per meter of root, a higher percentage of infected root tips, and higher inoculum efficiencies at all sample times than did plants grown in soil infested only with *V. dahliae*.

The average length of potato root colonized by *V. dahliae* was significantly greater 5 ($P < 0.01$) and 7 ($P < 0.09$) weeks after planting in roots grown in soil infested with *V. dahliae* and *P. penetrans* (0.13 and 0.27 cm of *V. dahliae*/m of root, respectively) than in soil infested with *V. dahliae* alone (0.05 and 0.12 cm of *V. dahliae*/m of root, respectively) or *V. dahliae* and *P. crenatus* (0.02 and 0.11 cm of *V. dahliae*/m of root, respectively) (Fig. 3E). Generally, the length of root colonized by *V. dahliae* was less than 1.0% for any one plant and often less than 0.5% across all treatments.

After 5 and 7 weeks, an average of 58.3 and 100%, respectively, of basal stem segments of plants grown in soil infested with both *V. dahliae* and *P. penetrans* were colonized by *V. dahliae* (Fig. 3F). Of those plants grown in soil infested with *V. dahliae* only, 0 and 75% were colonized after 5 and 7 weeks, respectively, and in soil infested with both *V. dahliae* and *P. crenatus*, 8.4 and 58.4% were colonized after 5 and 7 weeks, respectively. Thus, relatively low percentages of root tip infection and root colonization resulted in a high number of vascular stem infection by *V. dahliae*.

There were no significant differences ($P > 0.05$) in the number of nematode vermiforms per meter of root among treatments with nematodes 5 weeks after planting. On the average, there were 2.6 to 2.9 nematode vermiforms/m of root and approximately 176 to 233 nematode vermiforms per root system. Nematode vermiforms appeared to be randomly distributed among roots, with a few root segments containing 2 to 10 in close proximity. The initial density of nematodes added at planting (25 vermiforms/100 cm^3 in 1,000 cm^3 of soil = 250 vermiforms/pot) was almost recovered from roots 5 weeks after planting.

DISCUSSION

Initial infection and colonization of potato roots by *V. dahliae* occurred in undifferentiated tissues at the root tip and in the zone of elongation behind the root tip. This process was not visibly altered by nematode infection. Infection of the root cap and undifferentiated tissue also has been reported in cotton by *V. albo-atrum* (13) and in potato (cv. Russet Burbank) by *V. dahliae* (36). In these reports, as in our study, when successful infection occurred, the hyphae grew through the cortex towards the young, developing vascular tissues and along the endodermis surrounding the stele. Growth of *V. dahliae* through the cortex has been reported to proceed both inter- and intracellularly (13,43). We observed hyphae to be directed towards the vascular tissues and the vast majority of hyphae were concentrated internally growing along the endodermis. Hyphal densities in cotton also were lower near the root surface and outer cortex than near or adjacent to the stele, consistent with growth of hyphae from the root surface, through the cortex, and toward the stele (15,16). Hyphal growth along the endodermis parallel to the stele also has been reported (13,16,43).

Penetration into the young, developing xylem was observed (well before the first root hair in some instances) to occur by hyphal growth through pits in cell walls. Hyphae have been reported to penetrate the endodermis and enter the xylem vessels through pits in cell walls of cotton (13). Hyphae then grew within the xylem elements and transversed cell walls into adjoining vessels through pit apertures as described for infection of cotton (13,43) and potato (35) roots. Although we did not observe conidia in the vascular system, others have reported that conidia move with the transpiration stream (43). Xylem end plates act as conidial trapping sites (1,34), at which point the conidia germinate and the end plates are transversed by hyphal growth (13,43). In this manner, *V. dahliae* colonizes root vascular systems between the point of vascular infection and the stem (22). The presence of conidia in vessels of plants infected by vascular wilt fungi has been reported commonly, and the rapid colonization of the xylem of aboveground plant parts appears to be accomplished by conidia (43). In peppermint, when *V. dahliae* reached the xylem, its subsequent growth was mainly in the tracheae, until the latter stages when it invaded other tissue cells (33). We also observed extensive colonization of the vascular tissues before appreciable colonization of the cortex.

In many instances, colonization of the root vascular system was blocked by a visible host response, often near the root tip or just behind the zone of elongation, but distal from root tips as well. The host response consisted of dark brown (necrotic) cells almost always confined to the vascular cylinder. The response was in almost all infected root tip segments, regardless of the presence of nematodes. The dark brown material, which may have been intensified or altered in color or consistency by the staining technique, may result from the accumulation of gums, callose, lignin, suberin, or phenols (13,34,35,43). This response may serve to restrict the movement of *V. dahliae* further into the vascular system. Hyphae of *V. dahliae* were never observed in distal portions of the root past this barrier. This would not preclude, however, the formation and movement of conidia within vascular tissues (43), which may have slipped past this barrier and continued colonization of a portion of the root beyond the sample segment being observed. In roots in which further development of *V. dahliae* in the root tip or young vascular system was stopped, hyphae fully colonized the root tip resulting in loss of meristematic activity. Loss of meristematic activity in infected cotton roots also has been reported (13).

Many published reports describing the infection and colonization of roots of cotton (10,15,19,20,22), peppermint (33), potato (14,21), and other plant species (9,10,11,20,28) by *V. dahliae* have detected fungal colonization by placing or embedding roots on or in an agar medium. Distinct, localized colonies of *V. dahliae* grew from cotton roots placed on a growth-restrictive medium (22). The colonies on cotton and other plant species were described as superficial, small (only a few millimeters in length), and randomly scattered along the root (9,22). No colonization of the root by *V. dahliae* was reported within the first millimeter of the root apex; thus, the initial point of contact by *V. dahliae* was suggested to be well behind the region of undifferentiated tissues. The frequency of recovered colonies and the colony length was reported to increase over time and with distance from the apex and covered larger portions of the root (16,22). These distinct, epiphytic colonies on or in the roots have been described as invasion sites, each representing distinctly different sites of penetration (28).

Our observations did not support the hypothesis that vascular infection of roots takes place through differentiated tissues. In our studies, *V. dahliae* grew through undifferentiated tissues in the zone of elongation and then entered young xylem vessels. Vascular infection of roots occurred early and this was the norm. It is not clear whether the host response we observed was elicited by entry of the fungus into the vascular system or by initial infection in the root tip. Since the host response was confined to the vascular cylinder, our working hypothesis was that *V. dahliae* infects the vascular system early and regularly, and only then does the host respond. This hypothesis deserves further study since it may be relevant to host resistance mechanisms and interactions between nematodes and other organisms.

In our studies, we did not observe discrete, epiphytic, cortical colonies of *V. dahliae* on potato roots as described for *V. dahliae* on cotton (22). Colonies of *V. dahliae* grew from potato roots when placed on a semiselective medium; but the existence of discrete colonies in the cortex of potato roots was not supported using the immunoenzymatic assay to visualize hyphae of *V. dahliae*. Root extension after infection was not observed in our studies. In our proposed root colonization model, vascular infection by *V. dahliae* occurs early in developing vascular tissues, and conidia are formed and then transported with the transpiration stream (43). Further colonization of the vascular system occurs at a point distal to the root tip depending on the location of conidial trapping sites (1,34). Cortical colonization then occurs by hyphal growth from the vascular system into the cortex. This may result from an increased presence of *V. dahliae* in the vascular system over time, less resistance in vessel elements to lateral spread (34), and less resistance in cortical cells because of the disruption of physiological processes with vascular infection. Thus, colonies observed growing from the cortical tissues of roots into agar medium may be the result of hyphae growing out from the vascular system, not growing in through the cortex. The study of infection by Perry and Evert (35) supports our conclusions when they state "...the hyphae ramified throughout the tracheary elements and xylem parenchyma, invaded the phloem, and eventually moved out into the cortical region...." More detailed comparative studies are needed to reconcile the differences in infection patterns observed in our studies and those described in cotton (9,22).

Results of our experiments did not support the hypothesis that feeding injury by root-lesion nematodes provides a direct avenue of entry of *V. dahliae* into the root system. The physical act of nematode feeding on plant roots was not spatially related to entry of *V. dahliae* into roots and vascular tissues. Stained hyphae of *V. dahliae* were not observed in areas of cortical cell disruption or necrosis that resulted from nematode activity, regardless of the *Pratylenchus* species involved.

Initial infection of roots by *V. dahliae*, however, was influenced by nematode activity in that root tips became infected to a greater extent in the presence of nematodes compared with treatments without nematodes. This enhancement of initial infection was not *Pratylenchus* species-specific, nor did it appear to be spatially related to nematode feeding sites. Nematodes were usually not observed in the area of the root tip where infection by *V. dahliae* occurred, and may or may not have been present on the same root. Because roots were cut during sampling, nematodes may have been present in *V. dahliae*-infected roots, but this could not be determined.

Several alternative hypotheses were consistent with these observations. Nematode feeding may increase root exudation and increase rhizosphere width, which in turn may increase the number of root contacts with microsclerotia and result in a higher percentage of root tips infected. Nematode activity also may stimulate root branching, which would have the same effect and increase the number of root tip-microsclerotium contacts. A higher number of root tips per meter of root was observed 5 weeks after planting in treatments with nematodes as compared with those without.

Increased root tip infection by *V. dahliae* in the presence of nematodes did not, however, explain the observed species-specific interactions in PED, primarily with *P. penetrans*. Increased vascular colonization by *V. dahliae* of both roots and stems was observed in the presence of *P. penetrans*, compared with treatments with *V. dahliae* alone or in combination with *P. crenatus*. *P. penetrans*, but not *P. crenatus*, may affect the physiology of the

potato plant in some way that promotes root and stem colonization of the vascular tissues by *V. dahliae*. The absence of a physiological response in the root tip and the timing of any response may be a factor for entry of *V. dahliae* into the vascular system, and this may be specifically mediated by activity of *P. penetrans* in the root.

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