BARD

FINAL REPORT

PROJECT NO. US - 1501 - 88

MECHANISMS OF INTERACTION BETWEEN *VERTICILLIUM DAHLIAE* AND ROOT-LESION NEMATODES IN THE POTATO EARLY DYING SYNDROME

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1993

4106F

Appendix VI

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Date: August 26, 1993

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BARD project No. US-1501-88R

Title

Mechanisms of interaction between Verticillium dahliae and root-lesion nematodes in the potato early dying syndrome

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Project's starting date: October 1, 1989

Type of Report: 1st Annual _____ 2nd Annual _____ Final X

Signature
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Signature
Institution's Authorizing Official

FINAL REPORT

BARD Grant No. US-1501-88R
October 1, 1990 - January 31, 1993

MECHANISMS OF INTERACTION BETWEEN VERTICILLIUM DAHLIAE AND ROOT LESION NEMATODES IN THE POTATO EARLY DYING SYNDROME

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ABSTRACT

Studies in both the field and under controlled conditions confirmed the importance of root lesion nematodes, both *Pratylenchus penetrans* (Pp) and *P. mediterraneus* (Pm), in the development of potato early dying. Pm was shown to be able to complete its life cycle on potato roots both as an ecto- and as an endoparasite. In petri dish studies, more than twice as many Pm were found to move toward nematode-infected roots than towards uninfected roots. Exudates from nematode-infected roots were shown to affect sporulation by *Verticillium dahliae* (Vd) at the root tip. These exudates seem to contain a stable material that can trigger sporulation of Vd in a root-free system. Histological studies supported the hypothesis that Pp can interact with the potato plant to promote root and stem colonization of vascular tissues by Vd, even though root tip infection by either Pp or *P. crenatus* (Pc) may be similar. Root infection by Vd generally was very low (0.13-0.71 infected root tips/m of root) and increased only slightly over time in the presence of either Pp or Pc. There was a higher average number of root tips infected with Vd per meter of root in the presence of either Pp or Pc than without nematodes. However, the average length of root colonization by Vd was greater in the presence of Pp than with Pc or without nematodes. Even though both Pp and Pc were present within roots in comparable numbers, colonization of roots by Vd was increased only in the presence of Pp. These results indicate that any interaction on potato between Vd and *Pratylenchus* spp. takes place early in the life of the plant, prior to the time Vd has colonized the base of the stem.

OBJECTIVES OF ORIGINAL PROPOSAL

Objective 1. To study the effects of root tissue disruption by *Pratylenchus* species on activity of *Verticillium* microsclerotia in the rhizosphere and penetration phenomena at the root surface.

Objective 2. To study behavior of both pathogens and concomitant host tissue changes during interactive colonization in the early stages of infection following root penetration.

Objective 3. To elucidate the effects of *Pratylenchus* infection on host hormonal balance and response to *Verticillium* phytotoxins and the relationship of these to development of the PED syndrome.

INTRODUCTION

Where potatoes (*Solanum tuberosum*) have been in long-term production, premature vine death and declining yields are often a problem. In severe cases, early maturity of an entire crop can occur. This syndrome, called potato early dying, is the major limiting factor to commercial potato production in many areas of the United States and in the Negev Desert of Israel. The effect of early dying on yield is highly variable. Yield reductions as high as 30% have been documented from commercial fields in the USA (24) and reductions of 30-45% have been noted in Israel (16).

Detailed microplot studies on early dying conducted in the USA in Ohio (25) and Wisconsin (18) have conclusively demonstrated under field conditions that the disease there is caused by the interaction of *Verticillium dahliae* (Vd) and the root lesion nematode *Pratylenchus penetrans* (Pp). These studies have also shown that *P. crenatus* (Pc), another commonly-occurring nematode species, does not interact with Vd in this syndrome (23). In Israel, Vd is also the primary pathogen, but critical field and greenhouse studies have confirmed the involvement of the root-lesion nematode *P. mediterraneus* (Pm), formerly *P. thornei* (15). *Pratylenchus penetrans* does not occur in Israel.

Control of potato early dying in commercial fields is difficult. Although soil fumigation has been an effective management tool in many areas, treatment costs are high, and future use is threatened by increasing government regulation arising from health and environmental concerns (24). The use of disease resistant potato cultivars is the most practical method of control. Potato clones with high levels of resistance to *Verticillium* are being developed in both countries, but market acceptance of clones thus far available has not been widespread. Research in Israel has been directed towards the use of serological methods to detect Vd in locally produced seed tubers and the utilization of an extracellular toxin produced by the fungus in determining resistance to Vd (21). Until acceptable resistant cultivars become available, control of potato early dying must rely on integrated disease management systems involving crop rotation, site and cultivar selection, irrigation and nutritional management and, in some cases, soil fumigation.

The objectives of the research conducted in these studies were to obtain preliminary information on the mechanisms involved in the interaction of *Pratylenchus* species with *Verticillium dahliae* in the potato early dying syndrome. Work in Israel, which focused on *P. mediterraneus*, was designed to demonstrate and measure the interaction of this nematode with Vd. In this regard, it was essential to study first the life history and the behavior of the nematode on potato. Whole-plant studies then sought to quantify the interaction and finally to explain nematode effects on *Verticillium* at the rhizosphere level.

Studies in the USA took a histological approach, focusing on aspects of infection and colonization of potato roots by *Vd* where the presence of nematodes feeding on the roots might influence fungal activity. Two *Pratylenchus* spp. were studied for comparison; *P. penetrans*, which has been

shown to interact with *V. dahliae* in the disease and *P. crenatus*, which does not. Studies were designed to test two hypotheses. First, if root injury merely removes barriers for fungal entrance, then Vd should colonize roots to the same degree regardless of whether Pp or Pc is involved. Alternatively, Pp and Pc may alter host physiology differentially, thus affecting rates of root colonization and/or altering root exudates and thus the stimulation of microsclerotial germination in the rhizosphere.

STUDIES ON THE ROLE OF PRATYLENCHUS MEDITERRANEUS IN POTATO EARLY DYING

Materials and Methods

FIELD STUDIES - Field experiments were carried out at the Gilat Experiment Station in the northern Negev, Israel, during the fall seasons (planted in late August, harvested in January) of 1990 and 1991 and the spring seasons (planted in late February, harvested in June) of 1991 and 1992. The climate is arid-Mediterranean with mild winters and hot, rainless summers. Mean temperatures typical of these growing seasons are: Fall, 30-32C declining to 15-20C; Spring, 15-18C increasing to 28-30C. The soil is loessial, silt loam with an average of 5% organic matter and a pH of 7.8-8.1. Potatoes were raised in accordance with local practices. Two cultivars were used: Nicola (susceptible to Vd) and Alpha (tolerant to Vd). The trials were irrigated by overhead sprinklers to 700-800 mm of water. Fertilizers were incorporated into the soil preplant and additional fertilizer was injected into the irrigation system as needed. Standard pest and disease management practices were used to reduce the development of organisms outside the scope of this study as complicating factors.

All studies were conducted in experimental fields which were well infested with both Vd and Pm. Four years prior to these studies, potato stems well colonized with microsclerotia of Vd were incorporated into the soil. The field was then planted to the potato cultivar Spunta, which is highly susceptible to Vd and thus served to increase the fungal pathogen. The following year the field was planted to wheat which served to increase the nematode populations. In the two years prior to this study, potatoes were again grown as part of other experiments.

Populations of Vd (CFU/g soil) and Pm (individuals/100g soil) in experimental fields prior to application of treatments were:

Season/Year	Vd	Pm
Fall 1989	22.4±2.1	24±4.0
Fall 1990	19.6±0.9	180±56
Fall 1991	58.8±0.9	230±65
Spring 1990	40.0±10.0	98±12
Spring 1991	12.0±9	156±317
Spring 1992	64.0±2.3	190±42

Treatments were:

Vd and Pm infection	-	no treatment.
Vd infection only	-	application of metham-sodium (Vapam at 250 l/ha) or aldicarb (Temik at 15 kg/ha).
Pm infection only	-	two applications of benomyl at a concentration of 0.5% by spraying the foliage.
No infection	-	fumigating the soil with methyl-bromide at 500 kg/ha or metham-sodium (Vapam at 800 l/ha).

Densities of Vd were monitored by direct plating of soil samples on a selective medium; those of Pm by extraction of nematodes from soil and counting. All treatments were evaluated visually for maximum development of early dying symptoms on a subjective rating scale of 0-5 (0=no visible symptoms and 5=severely diseased plant). An average disease index was then calculated. Stem sections were excised from the top nodes of sampled plants and the concentration of Vd in the stems was determined by plating of macerated tissue. At harvest, tubers were dug from 2-m long sections of row and yields were calculated in grams of tubers per plant.

CONTROLLED INOCULATION STUDIES - Potato plants (cv. Nicola) for controlled inoculation studies were obtained from "eyes" placed on moist filter paper for 3-5 days until roots appeared. Rooted sprouts were then planted in dry-heat-sterilized sandy loam soil or a mixture of sandy soil plus peat and vermiculite (1:1:1). Seedlings of common vetch (*Vicia sativa* cv. Yovel) and wheat (*Triticum aestivum* cv. Bet-Lehem) were obtained from seeds germinated on moist filter paper and planted as above.

Inoculum of *P. mediterraneus* was maintained in monoxenic cultures on alfalfa callus on STW medium (27) enriched with 2 ppm of 2,4-D. The cultures were kept in the dark at 18C. For inoculation purposes, nematodes were extracted from 10- to 12-wk old cultures using a Baermann funnel. Generally 8,000-10,000 individuals (vermiforms) were recovered from each culture dish. The nematode suspension was then diluted with water to a known concentration and the inoculum then mechanically mixed with test soils to obtain the desired nematode density.

Cultures of *V. dahliae* were maintained on PDA. Inoculum of the fungus was obtained by growing it in flasks containing Chapek's medium (32g/l) and incubating them on a shaker for 4-5 days at 27 C. Resultant conidia were diluted to the desired inoculum concentration and mechanically mixed with test soils. In post planting inoculations, conidial suspensions were applied to the soil as a drench. In cases where microsclerotia were used for inoculation, the amounts of inoculum added were determined by weight.

All experiments were conducted in 80-ml styrofoam containers incubated in controlled environment chambers set for 8h light at 20 C and 16h dark at 16 C, to simulate fall growing season conditions. The plants were fertilized with a commercial mixture (20-20-20) and watered sufficiently to keep the soil

moist. Under these conditions most experiments lasted 3-4 weeks. Plant growth parameters were determined by measurements of shoot height and fresh and/or dry weights of shoots and roots. Visual disease symptoms were evaluated using the 0-5 evaluation scale and a disease index was calculated, as was done for field studies.

Nematode populations in roots were determined by recovering nematodes from the roots employing Youngs' incubation and Baermann funnel techniques, and counting them under a dissecting microscope.

The extent of infection by *V. dahliae* was determined by incubating 5-mm potato stem slices for several days in petri dishes containing 1% water agar and counting the fungus colonies on their surfaces. Where data is reported as colony-forming units (CFU) per cm of stem, stem sections were macerated in distilled water and the resultant suspension dilution-plated on a selective medium.

Several controlled inoculation studies were conducted at the Volcani Center, Bet Dagan, and at the Gilat Experiment Station in the Northern Negev. A study at Bet Dagan was designed to evaluate the quality of potato as a host of *P. mediterraneus*, as compared to common vetch and wheat. Test soils were infested with Pm at the rate of 700 vermiforms per pot on the day of planting and then each pot was planted with a single plant. At 48- to 72-hr intervals for a period of 20 days, nematode populations were determined within the root systems of four plants that were sampled for each host. This experiment was repeated three times with minor variations. Several experiments were also done at both locations to examine the effects of inoculation with one or both pathogens on potted plants and the optimal conditions for enhancement of *V. dahliae* invasion, colonization, and reproduction within the potato plants.

EXCISED ROOT STUDIES - Excised roots used for *in-vitro* experiments, were obtained from potato plantlets grown under aseptic conditions on modified Murashige and Skoog (MS) medium (20). The excised roots were then cultured on Skoog, Tsui and Whites' (STW) medium (27). Micro-Baermann funnels were used under aseptic conditions to obtain nematode inoculum free of other microorganisms for use in these experiments. Inoculations were done in a manner similar to those discussed in the previous section.

Excised roots used in histological studies were cleared for 2 min with 1% sodium hypochlorite, and stained with acid-fuchsin for 4-6 days at 50 C before examination. Light microscopic observations of *P. mediterraneus* behavior were done using a monoxenic nematode culture on excised potato roots. Scanning electron microscope (SEM) observations were made on various objects fixed and processed following conventional procedures. JEOL SEM at 10 kv was used throughout.

Results

ENHANCEMENT OF POTATO EARLY DYING IN THE FIELD - The following table summarizes the data collected during the field studies conducted at Gilat:

Potato Cultivar:		NICOLA			ALPHA		
Season/Year	Treat[1]	DI[2]	Vd[3]	Yield[4]	DI	Vd	Yield
Fall 1990	V+P	4.8a	2540a	219c	3.0a	1400a	820a
	V	3.1b	407b	422ab	1.7b	990b	950a
	P	1.1c	33c	708a	1.5b	800b	800a
	None	0.5c	10c	636a	0c	0c	918a
Fall 1991	V+P	5.0a	4690a	300b	3.5a	2600a	740a
	V	3.5b	677b	546ab	2.0b	1000b	800a
	P	2.8bc	528b	812a	1.9b	450b	720a
	None	0.8c	33c	789a	0c	0c	800a
Spring 1991	V+P	3.8a	416a	640b			
	V	3.0a	560a	680ab			
	P	2.0ab	330ab	660b			
	None	0	10c	920a			
Spring 1992	V+P	4.5	892a	560b			
	V	3.2	680b	640b			
	P	2.1	400b	900a			
	None	0	50c	1080a			

Footnotes:

1. Treatments: V+P = Vd+Pm, V = Vd only, P = Pm only, None = no pathogen.
2. DI=Disease Index. Plants evaluated using the scale 0=no visible symptoms, 5=severely diseased plant.
3. Vd populations in upper stem. Number is CFU/cm of stem.
4. Yield in grams of tubers per plant.

With the susceptible cultivar Nicola, infection with Vd resulted in striking early dying symptoms and a massive reduction in yield during the Fall season, especially if Pm was also present. Effects in the Spring season were much less evident and were not enhanced by co-infection with the nematode. With the *Verticillium*-tolerant cultivar Alpha, tests in the Fall season resulted in neither pathogen having a significant effect on yield, although infection with Vd did result in some symptom development. With Nicola, *Verticillium* colonization in the plant xylem was increased between 4-100 fold when Pm was also present. Even with Alpha, there was some increase in Vd recovered in the presence of Pm.

COMPARISON OF POTATO WITH VETCH AND WHEAT AS HOSTS OF P. MEDITERRANEUS - The ability of *P. mediterraneus* to reproduce on potato as compared to common vetch and wheat was studied in experiments carried out in controlled environment chambers. Populations of Pm per gram of roots of each of the host plants at various time intervals following inoculation were as follows:

Host Plant	Days: 2	4	6	8	10	12	14	16	18	20
Vetch	78	93	210	230	600	740	1380	1270	1650	2760
Wheat	85	95	250	420	500	480	530	610	950	1300
Potato	60	83	170	240	420	510	470	430	520	470

Two distinct phases were observed in development of the nematode population on these hosts. During the first 10 days following inoculation, nematode populations within the roots of all three host plants increased similarly to approximately 500 vermiforms per gram of root fresh weight. During the following 10-day period, however, populations of Pm in roots of common vetch increased 5-fold, whereas in wheat only 2.5-fold, and in potato hardly at all. Essentially the same results were observed all three times the experiments was conducted. No differences are evident between the three hosts during the first stages of the nematode-host relationship, i.e. attraction, invasion of the roots, and establishment of the infective stage within root tissues. However, in the second phase, when the reproduction processes of the population took place, the three hosts behaved differently: common vetch facilitating the most reproduction, wheat intermediate, and potato the poorest.

INTERACTIVE EFFECTS OF *P. MEDITERRANEUS* AND *V. DAHLIAE* ON DISEASE DEVELOPMENT - Experiments designed to evaluate the effects of co-infection with *P. mediterraneus* and *V. dahliae* were conducted with considerable variation. Studies at Bet Dagan explored the effects of preplant inoculation with either or both pathogens on the incidence of the fungus within potato stems. This was determined by observing fungal growth from excised stem sections plated on water agar.

In Experiment A, plants were transplanted into soil infested with 1000 Pm vermiforms/pot, 1.4 X 10^7 Vd conidia/pot, both, or neither. No differences were observed in the incidence of the fungus within the potato stems between the Vd and Vd + Pm treatments. In both treatments, Vd was recovered from 2/3 of the stems. Populations of the nematode were 4820 in the Pm treatment as compared with 7105 in the Vd + Pm treatment.

In Experiment B, inoculation of some treatments with Vd was delayed for various time periods following inoculation with Pm to detect any predisposition effect exerted by the nematode. Nematode inoculum was applied preplant in all cases at 1000 vermiforms Pm/pot. Vd was applied preplant and as a soil drench 2, 4, 6 and 8 days following planting at 1.4 X 10^7conidia/pot. Treatments included all combinations of Vd alone and in

combination with Pm. In this study, Vd was recovered from about half of the stems sampled from all treatments inoculated with the fungus. No predisposition effect was observed to be exerted by the nematode.

Experiment C was designed to test the effect of increasing nematode populations on the incidence of *V. dahliae* in the potato stems. Pm was added to soils preplant at 250, 500, 1000 and 2000 vermiforms/pot. Treatments including both pathogens were also infested with Vd by adding a suspension of 0.8×10^7 conidia/pot. Results showed that increasing the preplant nematode population did not increase the incidence of Vd recovered from potato stems. Nematode populations recovered from the roots of treatments inoculated with both pathogens were considerably higher than those recovered from the treatments inoculated only with nematodes. This experiment was repeated including a lower nematode population of 100 vermiforms/pot in addition to the previous treatments. Again, no influence on the incidence of Vd recovery from potato stems was found. As before, nematode population levels recovered from potato roots inoculated with both pathogens were higher than from those inoculated with nematodes alone.

Experiment D tested the influence of increased amounts of Vd inoculum on the recovery of the fungus from potato stems. In this study, the preplant population of Pm was held constant at 1000 vermiforms/pot and Vd was added at rates of 0.9×10^8, 0.9×10^7, 0.9×10^6, and 0.9×10^5 conidia/pot. All combinations of these rates were tested. Results showed that increasing the amount of Vd inoculum did not increase the incidence of the fungus recovered from the potato stems. The nematode inoculation had a slight but insignificant effect on recovery of the fungus.

In Experiment E, variable amounts of inoculum of both pathogens were tested. In addition, microsclerotia rather than conidia were used in Vd inoculum. Treatments included either 500 or 1500 Pm vermiforms/pot and/or either 1, 2 or 4 mg of Vd microsclerotia/pot. All the combinations of the above plus an uninoculated control were included in the experiment. As before, the nematode populations had no significant effect on the incidence of fungal recovery from the potato stems. On the other hand, the nematode populations recovered from root systems in the treatments inoculated with both pathogens were considerably higher than in the treatments inoculated with Pm alone.

Experiment F was a split-root study designed to see whether the two pathogens had to be in physical contact with each other to have an interactive effect. Stems of potato plants were split at their bases to the height of 3 cm and were allowed to root in an aqueous mineral solution for 1 wk. Each rooted plant half then was planted in one of two pots, which were held together with a strong rubber band to form a single experimental unit. Soils in pots were infested preplant with either or both pathogens at rates of 1000

Pm vermiforms/pot and/or 4-5 mg Vd microsclerotia/pot. There were six replications per treatment. Treatments and results were as follows:

Left pot	Right pot	Recovery of Vd per 24 stem sections
-	Pm+Vd	10
Vd	Pm	5
-	Pm	-
-	Vd	6
Uninfested Control		-

Recovery of Vd from stems of plants where both pathogens were on the same root half was double that when Pm was on the other root half, or not present, indicating that both pathogens had to be in physical contact for the interaction to occur.

In Experiment G, done at Gilat, soil was used from Lahav which was naturally infested with Pm at 400 vermiforms/100cm3. This soil was autoclaved for use as the control and then a portion of the autoclaved soil was reinfested with Vd at 10^5 conidia/pot for the Vd treatments. The treatment with both pathogens consisted of a mixture of the naturally infested soil and the autoclaved soil that had been reinfested with Vd. There were three replications to the experiment and data were taken 40 days after planting.

Treatments and results were as follows:

Treatment	Pm from roots vermiforms/100cm3)	Vd from top node (CFU/cm)	Disease Index	Height (cm)
Control	0c	0b	0c	55c
Pm	690b	0b	0c	52c
Vd	0c	420b	2.0b	39b
Vd + Pm	1920a	31000a	4.0a	30a

Stimulation of early dying due to co-infection with Vd and Pm occurred in this study, as was evident by the significant increase in the average disease index value. Plant height was reduced by Vd, and there was some further stunting that resulted from co-infection with Pm. There was increased reproduction of Pm in the presence of Vd, as was seen in the Bet Dagan studies, and a considerable increase in the amount of Vd recovered from the upper part of infected plants.

LIFE CYCLE AND PATHOGENICITY OF *P. MEDITERRANEUS* ON EXCISED POTATO ROOTS - Studies were done at Bet Dagan on excised roots to investigate the mechanism of infection of *P. mediterraneus*. Movement of *P. mediterraneus* toward potato roots was studied by placing a 1- to 2-cm root section on one side of a petri plate containing 0.5% water-gelrite (an agar substitute) and a 1-wk old root section infected with *P. mediterraneus* on the opposite side. A

drop of nematode suspension, containing 30±5 vermiforms of Pm, was then placed at the center of each of ten replicate plates. Nematodes in the immediate vicinity of the root were counted daily. After 3 days, the number of nematodes around the uninfected roots, were 4.9±0.45 and around the infected roots were 10±3.6, more than twice as many. This experiment was repeated five times with similar results.

A second study was designed to allow observation of the development of Pm on potato root segments. Excised root segments 2-3 cm long were placed on half-strength STW medium in petri dishes. Twenty four hours later, a drop of *P. mediterraneus* egg suspension containing 15±5 eggs was placed in each of the dishes at a distance of 3 cm from the roots. Microscopic observations of nematode development and root infection were made daily.

Second stage larvae hatched from the eggs 2-7 days (most in 4 days) following inoculation. Nematode traces were observed all over the medium surface, but a higher density of traces were observed close to the roots **(Plate 1G)**. Some nematodes concentrated around the root tips, feeding on them as ectoparasites **(Plate 1A)**. In 2-4 days, those root tips ceased growing and became swollen with large hypertrophied cells on their surfaces **(Plate 1B,C)**. Some nematodes were feeding on epidermal cells and root hairs. In these cases, the entire feeding site became somewhat swollen and the epidermal cells became considerably hypertrophied **(Plate 1D,E)**. The root hairs being fed upon were damaged in two distinct manners. In certain areas the bases of the root hairs became extremely enlarged and spherical **(Plate 1H)**. In other locations, growth of the root hairs was severely curtailed by the feeding nematodes (Plate 1G) or the distal ends were abnormally branched **(Plate 1I)**.

As expected, the nematode also behaved as an endoparasite. A large proportion of the infective individuals invaded the root cortical parenchyma. At these locations the roots became somewhat swollen and the surfaces seemed to be coated with a slimy substance that presumably leaked from the injured tissues **(Plate 2A,B,C,D)**. The invaded section of the root was dark brown -a typical lesion symptom. The epidermis cracked in a few of the lesions, revealing various nematode developmental stages and the destroyed parenchymal tissue **(Plate 2D,E)**. Quite a few roots were completely covered by extensive lesions harboring all nematode developmental stages. On the other hand, many roots adjacent to the infected ones remained intact **(Plate 2C)**.

By the third week following inoculation, nematode larvae that remained outside the roots ceased feeding and stayed motionless beside the roots. At this stage the juveniles underwent molting, probably three consecutive molts, to produce adult females **(Plate 1F)**. No males were observed in the cultures. At four weeks following inoculation, eggs were laid. Many eggs were observed along the roots, presumably laid by females completing their life cycle outside the root, or by females migrating from lesions. Under the experimental conditions of this study, one life cycle of *P. mediterraneus* lasted approximately 4 wks.

PLATE LEGENDS

Plate 1. ECTOPARASITIC EFFECTS OF *PRATYLENCHUS MEDITERRANEUS* ON POTATO ROOTS.

A. Potato root tip attacked and fed upon by an aggregate of 2nd stage larvae.

B. Two to three days following attack, the root tip became swollen.

C. Large cells on the surface of the swollen root tip.

D. Nematodes feed on root epidermal cell.

E. Scanning electron micrograph of root fed upon by the nematode. Note the large epidermal cells.

F. *P. mediterraneus* undergoing molt process.

G. Curtailed root hairs caused by the nematode feeding. Note the traces of the nematodes on the medium surface.

H. Swollen root hair bases.

I. Branched root hairs.

Plate 2. ENDOPARASITIC EFFECTS OF *PRATYLENCHUS MEDITERRANEUS* ON POTATO ROOTS.

A. A discolored potato root harboring the nematodes.

B. An infected swollen root.

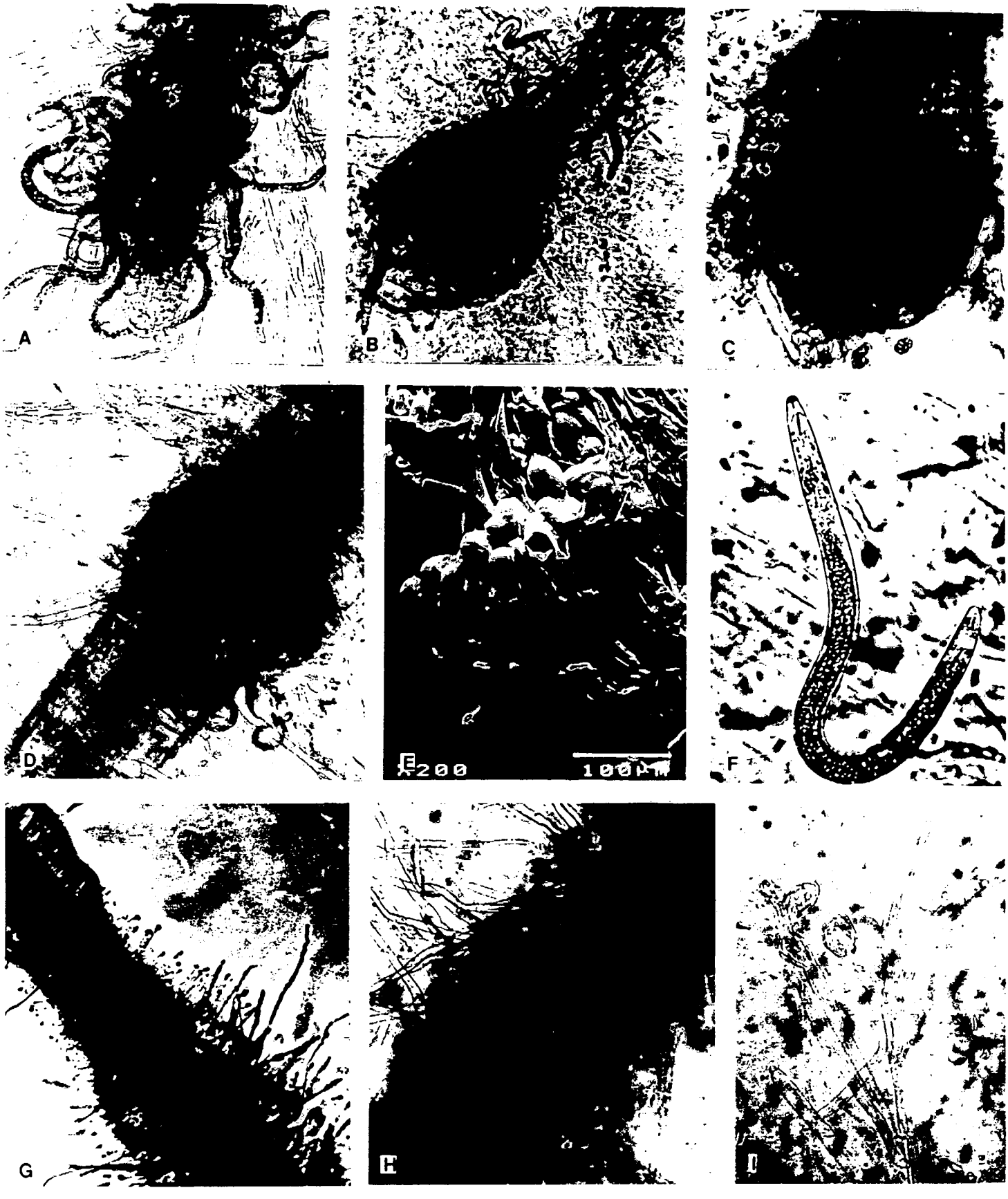
C. Intact root adjacent to infected root.

D. A scanning electron micrograph of a swollen lesion. Note the cracks in the epidermis and the coating on the lesion surface.

E. Higher magnification shows a nematode, an egg and the destroyed parenchymal tissue inside the lesion.

F. A scanning electron micrograph of a longitudinal section in a lesion.

G. A scanning electron micrograph through a section of a lesion showing bacteria associated with the nematode.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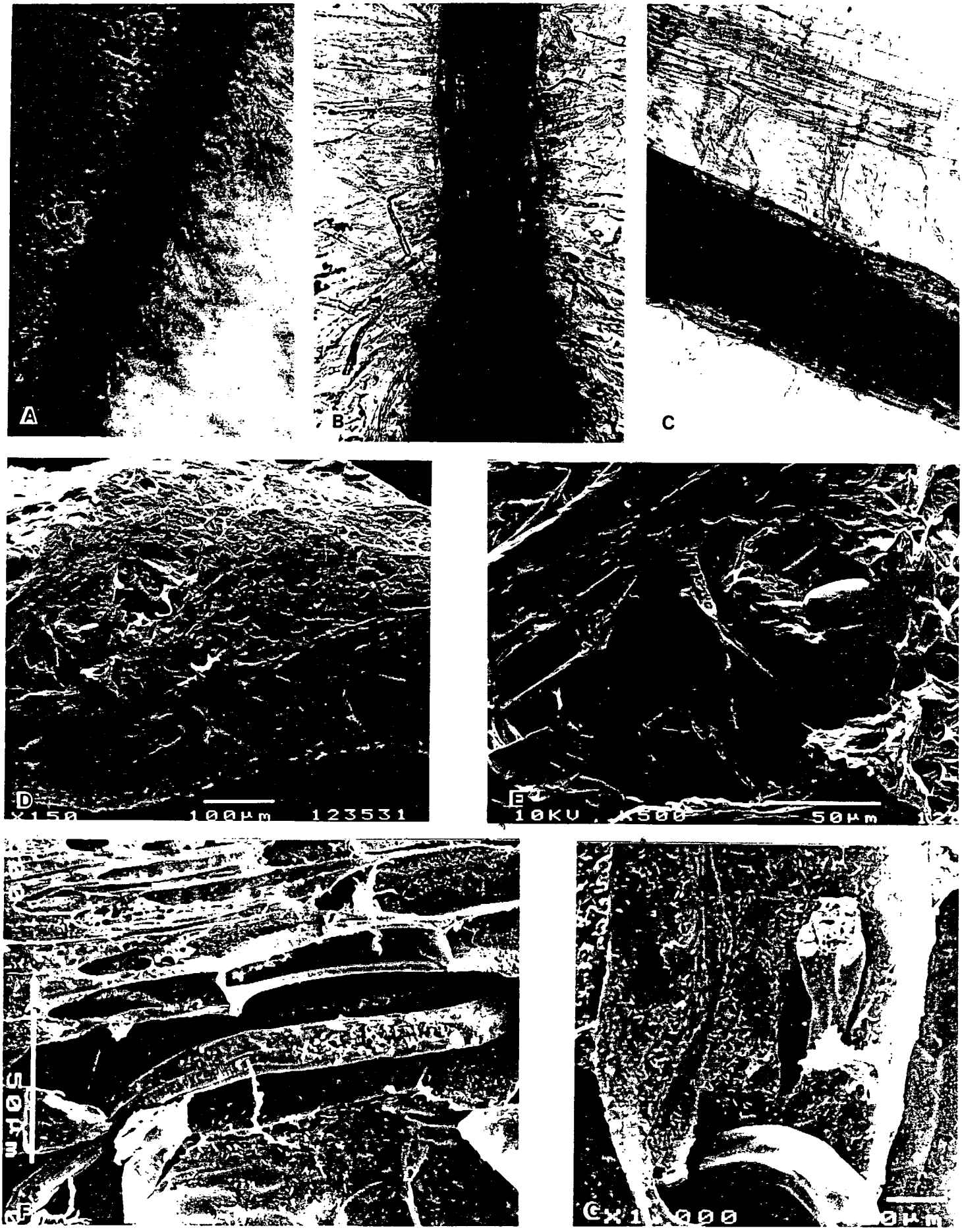
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EFFECTS OF *P. MEDITERRANEUS* INFECTION ON SPORULATION OF V. DAHLIAE IN THE RHIZOSPHERE - In certain SEM photographs made at Bet Dagan, it appeared that sporulation of Vd had occurred on the outside surface of root tips infected with Pm. This observation was in contrast to our previous working hypothesis that sporulation of Vd occurs mainly within the vascular system. In order to study potential interactions between Vd and Pm that might be occurring in the rhizosphere, studies were done at Gilat using sterile potato plantlets grown on MS medium. These were uprooted and replanted in a sterile mixture containing perlite and vermiculite (8:2 by volume) contained in 25-mm diam Pyrex test tubes covered with a clear plastic cup. Three to four days after transplanting, inocula of both pathogens were added to the tubes using sterile pipettes. Symptoms and populations of both pathogens were examined in root and stem tissues during the period of 14-30 days following inoculation. At the termination of the experiment, plants were removed from the perlite/vermiculite mixture, macerated, and the colony forming units of Vd were determined by dilution plating. Nematodes were extracted from root segments and counted. Treatments and results were as follows:

Treatment	Pm vermiforms/tube	Vd (CFU/plant)	Disease Index
Control	0	0	0
Pm	128	0	0
Vd	0	1860	1
Vd + Pm	678	20500	3

Results of this experiment were very similar to Exp G above. Disease symptoms were more severe when both pathogens were present. As in several previous studies, there was increased reproduction of Pm in the presence of Vd and a considerable increase in the amount of Vd recovered from the plants.

Using this system, further studies were done to see if root exudates from nematode-infected plants were affecting sporulation of Vd at the root tip. Tissue-cultured plantlets were grown in the sterile perlite/vermiculite mixture as above inoculated only with Pm. Plants were uprooted and roots of some plants were washed by dipping them three times in a reduced concentration of Hoglands nutrient solution and then finally in distilled water. Washing was done to remove residues of the culture medium and associated root exudates. Plants with both washed and unwashed roots were placed around the edges of a 9-cm sterile petri dish containing a Whatman #1 filter paper and 5 ml of distilled water. A drop of spore suspension of Vd was placed in the center of each petri dish. At the end of the experiment, the concentration of Vd within root tissues was determined. The difference in the final concentration of Vd was 4-fold higher in the non-washed root systems. These results indicate that some of the colony forming units of Vd measured by plating of extracts originated from propagules located outside the root system, presumably as a result of sporulation in the rhizosphere.

To examine this possibility further, sterile plantlets grown on a root-formation medium were transferred to a 9-cm sterile petri dish containing a Whatman #1 filter paper and 5 ml of distilled water. A piece of callus

containing Pm (0.5 cm^3) was placed on the top of selected individual roots, and the nematodes then were allowed to penetrate and infect these specific root systems thus inoculated. A drop of spore suspension of Vd was placed in the center of each petri dish. After 10 days of incubation, 1-cm diam squares of filter paper containing 1-cm lengths of root tips were cut out and removed. Each piece of excised paper and the associated potato root tip was suspended in a sterile phosphate buffer solution (pH 7.2) and then homogenized. Colony forming units of Vd were determined by dilution plating. The roots colonized with nematodes were heavily infected by both mycelium and spores of Verticillium (10.8×10^3 cfu Vd), whereas the roots that were not infected with Pm had much less colonization by Vd (350 cfu). This result confirms observations made by scanning electron microscopy indicating that sporulation of *Verticillium* occurred outside of the root tips that were infected with Pm.

Another experiment was set up to test the hypothesis that roots infected with Pm secrete materials which activate or stimulate sporulation of Vd within the rhizosphere. The experimental design was as above except that some roots were left uninfected by Pm as a control and no *Verticillium* inoculum was placed in the middle of the petri dishes. After a period of 10 days, 1-cm diam squares of filter paper surrounding the potato root tip zone were cut out and placed without the associated root tip on a different petri dish containing a spore suspension of Vd in distilled water. This system was incubated at 27C to allow spores, mycelia and/or microsclerotia to develop on the paper segments. After 5 days incubation, paper sections were homogenized as above and colony forming units of Vd determined. Paper segments that had been in contact with Pm-infected roots averaged 278 cfu of Vd, whereas those that had been in contact with uninfected roots averaged 12 cfu. These results indicate that root exudates from root tips infected with Pm contain a stable material that can trigger the sporulation of Vd in a root-free system. Our working hypothesis relative to this is that asparagine is connected to sporulation of Vd *in vitro* (1,4). In a preliminary study, Vd was grown in a liquid medium containing 0, 3, 5, 7, and 9mM of asparagine. After incubation, the log of the number of spores formed was 0, 0.7, 1.8, 3.9, and 3.8, respectively, indicating that asparagine stimulates *Verticillium* sporulation *in situ*.

A further experiment dealing with the influence of asparagine on sporulation of Vd used a system of excised potato roots that were not infected with Pm. Root tips were placed in a petri dish containing filter paper which had been pre-dipped in double distilled water containing several concentrations of asparagine. Since the asparagine was in distilled water in this study, the only source of carbohydrates, amino acids, and other nutrients for fungal growth were in root exudates lost from the root tips. Asparagine concentrations tested were 3, 5, and 10mM. When filter paper squares were cut out and placed on different petri dishes containing a spore suspension of Vd in distilled water, incubated for 5 days and assayed as above, log sporulation of Vd resulting from these treatments were 1.6, 2.7, and 3.0, respectively, indicating that asparagine had a very significant affect.

STUDIES ON THE ROLE OF PRATYLENCHUS PENETRANS AND P. CRENATUS IN POTATO EARLY DYING

Materials and Methods

PRODUCTION OF INFECTED HOST MATERIAL - Factorial experiments were conducted at Wooster, Ohio, in a greenhouse in 13-cm-diam plastic pots containing approximately 1000 cm^3 of an organic muck soil infested with two inoculum densities of *V. dahliae* (0 and 50 microsclerotia/cm^3 soil) and three populations of *Pratylenchus* (none, *P. penetrans*, and *P. crenatus*; each species at 25 vermiforms/100 cm^3 soil) with six replications per treatment per sample date. Plants were destructively sampled at 3, 5, 7, and 9 wk after planting. The isolate of Vd used throughout these studies was P-7 (VCG 4A) (13,14). Inoculum, consisting of Vd microsclerotia, was prepared as described previously (7). Inoculum of Pp and Pc were produced on monoxenic alfalfa callus culture also as described previously (19,22,25). Soil used in all experiments was a Rifle peat from northcentral (Celeryville) Ohio (7). Single-eye seedpieces (cv. Superior) obtained from virus-tested stock were used as planting material (25).

SAMPLING OF INFECTED ROOT MATERIAL - Entire root systems from each plant at each sample date were harvested by carefully washing the roots free of soil under a gentle stream of water in a large bucket. Root samples were taken using a modified grid method (10). The entire root system was placed on a glass plate in a shallow tray of water, and roots were teased apart with fine dissecting needles until they covered a specific area of the glass plate in a single layer. The glass plate with roots then was removed carefully from the tray and placed over a sampling grid consisting of 2 X 2-cm squares. Random selections of squares identified by five to eight colors each represented a separate 10% sample of the grid surface. 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 those areas of one color on the grid. Thus five to eight 10% root samples could be taken without disturbing or redistributing the roots. This insured a random sampling of roots without regard to root age, class, or order.

EVALUATION OF ROOT INFECTION - Initial attempts were made to evaluate cortical and vascular colonization of the roots by plating the root samples on or embedding them in a medium semi-selective for *Verticillium* (SPA) and then observing subsequent colony growth of Vd from the roots (5,11,17). This approach was based on a published technique used with other hosts of Vd. No Vd was ever observed growing from roots embedded in the medium, even after 5 wk of plant growth. Additionally, the medium always became colonized by many other fungi which may have inhibited growth of Vd in the medium. A further complication was that embedding the roots in the medium probably did not provide enough oxygen for fungal growth. Surface disinfesting the roots in low concentrations of NaOCl reduced the number of contaminating fungi, but did not improve recovery of Vd. *Verticillium dahliae* was recovered from surface plated roots, but contaminating fungi were still numerous. Thus, after considerable effort, this method was deemed unreliable and inefficient and was abandoned.

Further evaluations of cortical and vascular colonization of potato roots in these studies were done using an immunoenzymatic staining technique to visualize colonies of *V. dahliae* both in the cortex and in the vascular system (8,9). 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 that we prepared against a soluble protein extract of *V. dahliae* isolate P-7. This antibody serum was prepared according to the methods of Gerik et al (9). Root samples collected as described above were stored in a fixative solution until stained by this procedure.

For each sample, total root length was estimated using the line intersect method (28), and the length of root observed to be colonized by *V. dahliae* 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. Both uninfected and infected root tips were counted. A root tip was scored as infected when internally stained hyphae were detected and the data were expressed as the percent of infected root tips and the number of root tips per unit length of root. An additional 10% sample per plant was stained with acid-fuchsin (2) to facilitate visualization and enumeration of nematode populations in the roots in order to determine if the physical location of nematodes within tissues or their numbers affected the extent of colonization by Vd. These data also were used to determine if *P. penetrans* and *P. crenatus* infected potato roots equally. Using the same plants from which root samples were taken, vascular colonization of the stem was assessed over time by excising basal stem segments and plating portions on agar medium. After several days incubation, Vd could be observed growing out of infected vascular bundles, and thus the number of plants at each sample date which were colonized above the soil line by the fungus could be determined.

Results

Several qualitative assessments were made from these trials based on observations of treatments both with and without nematodes. Infection of roots by *V. dahliae* was observed to be almost entirely through the root tip or the area just behind the root tip in the zone of elongation. Discrete colonies of Vd in the cortex were not observed along the length of root as described for Vd on cotton (8,9,12), but rather the hyphae progressed into and colonized the vascular cylinder or was prevented from further development by a visible host response. Hyphae of Vd were not observed to be associated with nematode feeding or necrosis of cortical cells as a result of nematode feeding. This does not support the hypothesis that root injury as a result of nematode feeding facilitates entry of *V. dahliae* into roots.

A visible host response, consisting of dark brown cells (possibly due the accumulation of callose, lignin, and/or phenols), was observed in almost all infected root tips and some that did not contain visible hyphae of Vd. This response was almost always confined to the vascular cylinder, and thus could be distinguished from nematode injury, which was confined to the cortical cells. In some instances this host response was formed further back along the root and Vd was observed in the young vascular cylinder up to the

point of the host response. In these instances, the host response may have been weak or delayed and thus conidia of *V. dahliae* may have slipped past and further colonized the vascular system.

The presence of nematodes did not appear to be directly or spatially associated with vascular colonization by *V. dahliae*. Root tip infection and vascular colonization were observed 3 wk after planting, stem colonization was first detected 5 wk after planting, and symptom development did not occur until 8-9 wk after planting.

Quantitative data presented in this report are the average of two trials of the experiment (data was pooled to calculate averages). Each point in the figures per treatment per sample time represents the average of 12 observations (one sample per plant, six replicate plants per trial). A total of nine greenhouse experiments were conducted, but technical problems caused us to discard some of the data. Data here are illustrative of those in which environmental conditions were optimal for plant growth and pathogen activity, and the recovery of *V. dahliae from* basal stem segments 5-7 wk after planting assured us that the normal processes of pathogenicity were occurring.

Root length was comparable between the two trials. The number of infected (stained) root tips and the length of root colonized by *V. dahliae* was generally lower in the second trial than the first trial and differences were not as great among treatments. However, stem colonization by Vd generally was comparable between the two trials, thus indicating that the root colonization data was valid and that the fungal/nematode interaction was occurring in a natural manner.

The average rate of root growth per plant in the two trials generally was linear with root length increasing from an average of approximately 10-15 m after 3 wk to 54-85 m after 5 wk and 127-172 m after 7 wk for all treatments. There was a decrease in the average rate of root growth for plants growing in soil infested with both Vd and Pp between 5 and 7 wk after planting (**Fig. 1**) compared with the other treatments, and the lowest average amount of root per plant was observed in this treatment 7 wk after planting. The average number of root tips per meter of root varied widely at 3 wk after planting, but approached a constant after 5 and 7 wk, averaging about 16-20 root tips/m of root (**Fig. 2**) for all treatments. Potato root systems were extremely fibrous in this system. The vast majority of roots were laterals and averaged only 0.23 mm in diameter. There were only about ten larger, main roots per plant which originated from the basal portion of the stem. These averaged 0.5-1.0 mm in diameter and were capable of growing about 1.5 cm/day.

The number of infected root tips per meter of root increased slightly from 3 to 7 wk after planting in all treatments with averages generally in the range of 0.13-0.71 infected root tips/m of root (**Fig. 3**). This corresponds to the near constant production of root tips per meter of root and the linear increase in root length over time, and suggests that root tips become 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. 4**). On the average, the percentage of infected root tips was low, ranging from 0.7-3.0% for all treatments. The infection efficiency

of the inoculum was defined as that portion of the inoculum on or adjacent to the infection court which induced successful infection of the root (3). This was 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 in these studies was low, averaging from 0.014-0.060% for all treatments. Plants growing in soil infested with both Vd and Pp or Pc 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 growing in soil infested with Vd only. Nematode feeding as ectoparasites around the root tip may influence root exudates and/or increase rhizosphere width, but the phenomenon may be non-specific.

The average length of colonization of potato roots by *V. dahliae* was greater 5 and 7 wk after planting in roots growing in soil infested with both Vd and Pp (0.13 and 0.27 cm of Vd/m of root, respectively) than in soil infested with Vd alone (0.05 and 0.12 cm of Vd/m of root, respectively) or with both Vd and Pc (0.02 and 0.11 cm of Vd/m of root, respectively) (**Fig. 5**). The rate of colonization also appeared to be greater in the treatment with Pp. Generally, the length of root colonized by Vd was less than 1.0% for any one plant, and often less than 0.5% across all treatments.

After 5 and 7 wk, an average of 58.3 and 100%, respectively, of plants growing in soil infested with both Vd and Pp were colonized by Vd (**Fig. 6**). Of those plants growing in soil infested with Vd only, 0 and 75% were colonized after 5 and 7 wk, respectively, and in soil infested with both Vd and Pc, 8.4 and 58.4% were colonized after 5 and 7 wk, respectively. A relatively low percentage of infected root tips and a low amount of root colonized by Vd resulted in a high number of plants with in the stem.

There were no differences in the numbers of nematodes per meter of root among treatments with nematodes at 5 wk after planting (**Fig. 7**). On the average, there were 2.6-2.9 nematodes/m of root and approximately 176-233 nematodes per root system. Nematodes generally were randomly distributed (observation only) among roots with a few root segments containing 2-10 nematodes in close proximity. Interestingly, the initial density of nematodes added at planting (250 vermiforms in the 1000 cm^3 of soil contained in each pot) was almost recovered from roots 5 wk after planting. Even though both Pp and Pc were present in roots in comparable numbers, only *P. penetrans* increased colonization of roots by Vd, and this occurred with only low populations of Pp in the roots.

Fig. 1

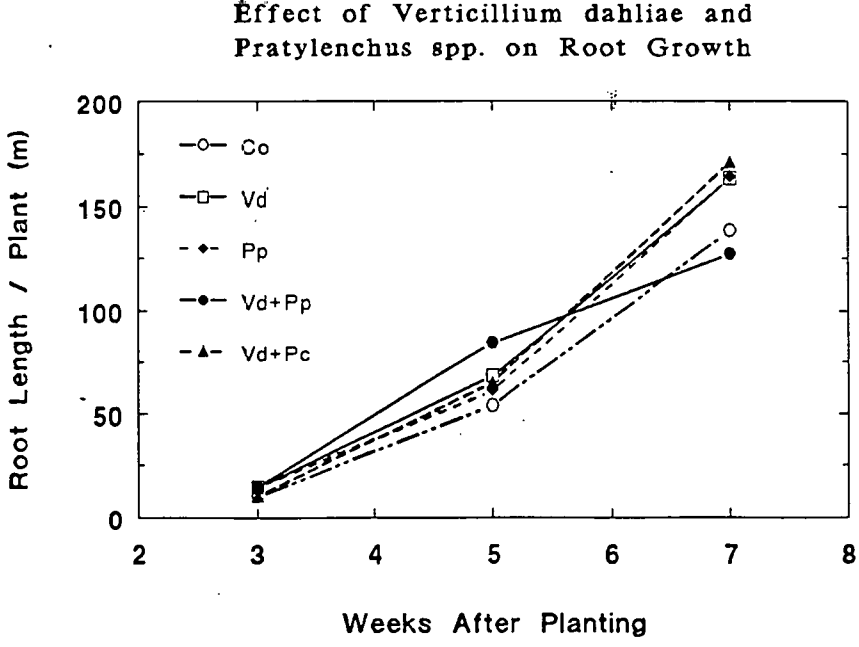


Fig. 2

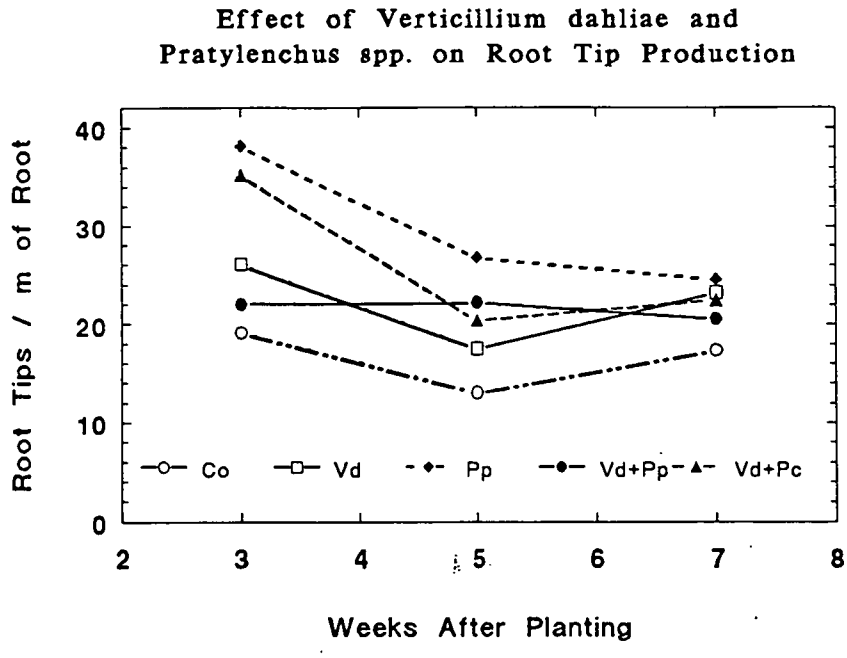


Fig. 3

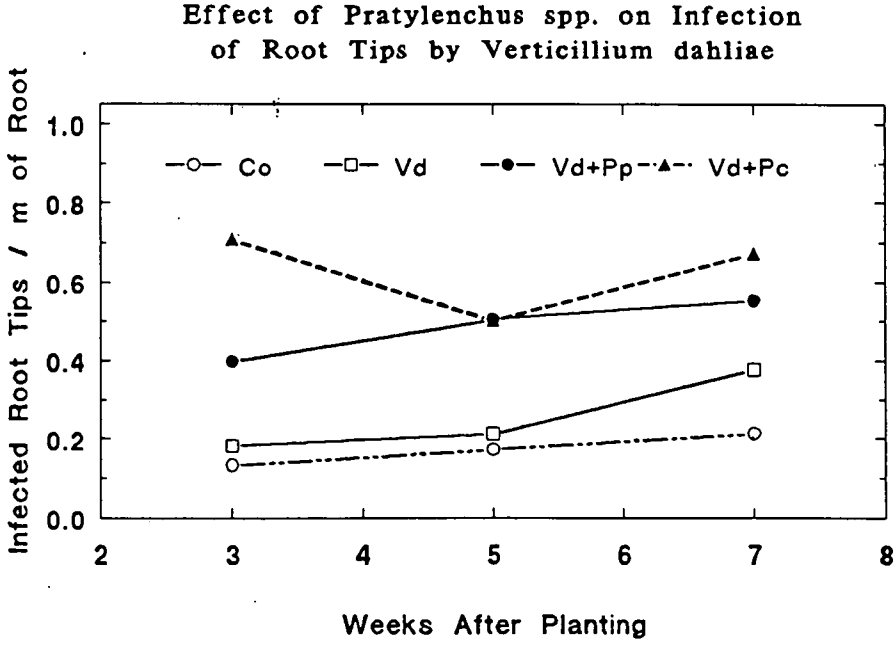


Fig. 4

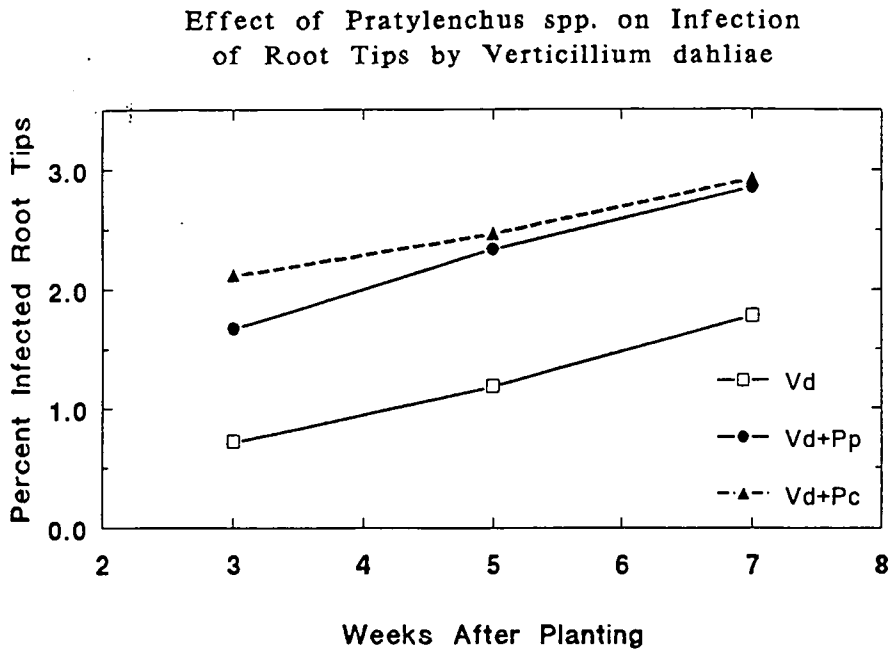

Effect of Pratylenchus spp. on Infection
of Root Tips by Verticillium dahliae
Percent Infected Root Tips
3.0
2.0
1.0
0.0
2
3
4
5
6
7
8
Weeks After Planting
Vd
Vd+Pp
Vd+Pc

Fig. 5

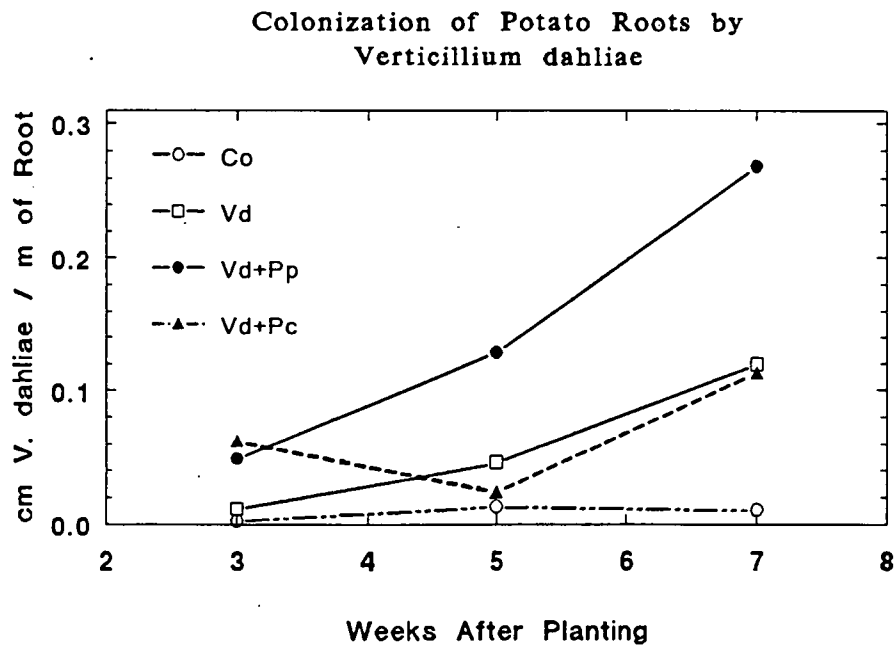

Colonization of Potato Roots by
Verticillium dahliae
cm V. dahliae / m of Root
0.3
0.2
0.1
0.0
2
3
4
5
6
7
8
Weeks After Planting
Co
Vd
Vd+Pp
Vd+Pc

Fig. 6

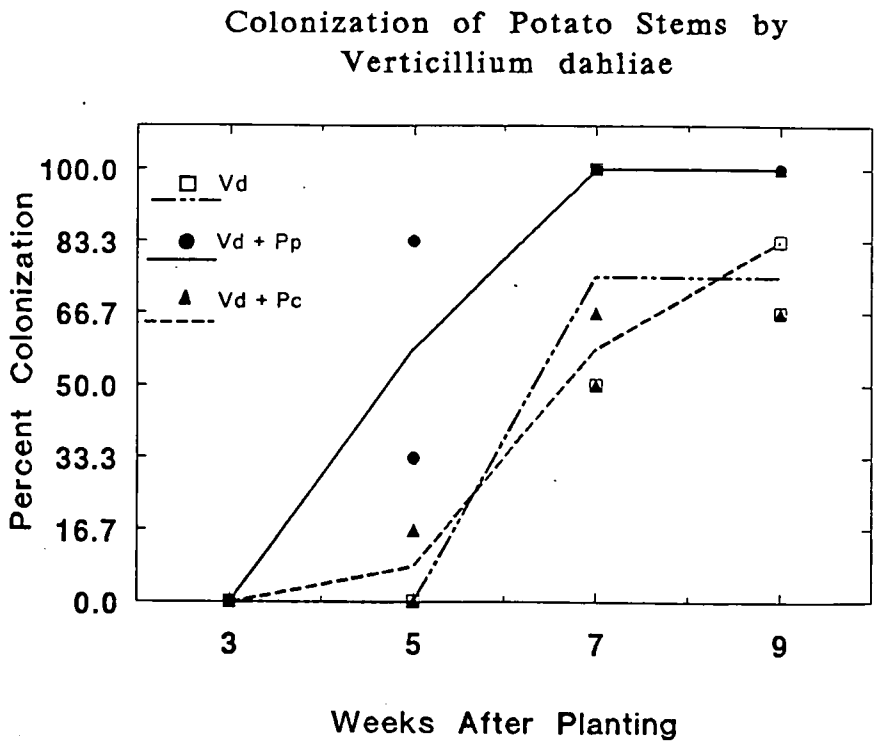
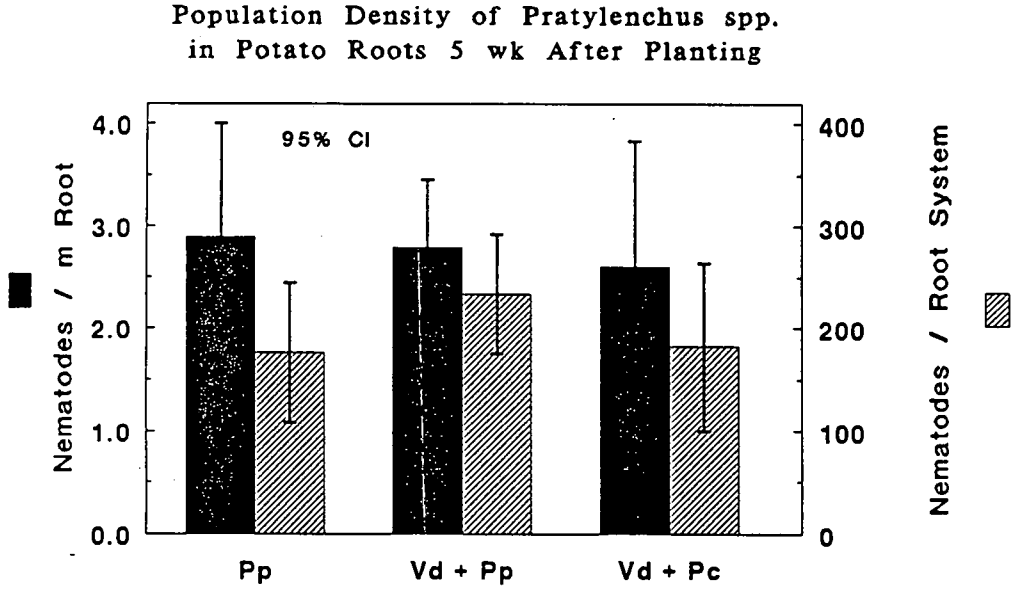

Colonization of Potato Stems by
Verticillium dahliae
Percent Colonization
100.0
83.3
66.7
50.0
33.3
16.7
0.0
Vd
Vd + Pp
Vd + Pc
3
5
7
9
Weeks After Planting

Fig. 7


Population Density of Pratylenchus spp.
in Potato Roots 5 wk After Planting
95% CI
Nematodes / m Root
4.0
3.0
2.0
1.0
0.0
Nematodes / Root System
400
300
200
100
0
Pp
Vd + Pp
Vd + Pc

DISCUSSION

Studies at both locations confirmed the importance of root lesion nematodes, both *Pratylenchus penetrans* in the USA and *P. mediterraneus* in Israel, in the development of the potato early dying syndrome. Close observations of the life cycle and feeding habits of *P. mediterraneus* on potato roots showed that Pm could complete its life cycle both as an ecto- and as an endoparasite, Although it could be argued that the behavior of nematodes under monoxenic conditions is very much different from their behavior under the natural conditions in the soil environment, the authors of this study, however, believe that the excised root monoxenic culture used here provides the nematode its essential requirement as an obligate parasite, thus enabling it to feed and then to develop and reproduce in its normal pace, much the same as occurs in the soil environment. Even though species of the genus *Pratylenchus* are considered to be typical endoparasites, there are indications that at least some *Pratylenchus* species can feed both as ectoparasites as well as endoparasites (29).

The damage observed to be inflicted by Pm feeding as an endoparasite agrees with many previous descriptions of various *Pratylenchus* species (30). However, there are no records documenting damage inflicted by *Pratylenchus* species as an ectoparasite. Damage observed here was quite severe, resulting in injury to the growth function of the root tip meristem, and the water and mineral nutrition absorptive capacity of the root hairs. It was not the intent of this study to provide quantitative data and analysis of the amount of damage the nematode could inflict as an ecto- and endoparasite. Most probably it would be impossible to draw a distinct line between the two modes of parasitism, but the ectoparasitic nature of *P. mediterraneus*, and probably of other *Pratylenchus* species, should not be overlooked.

In petri dish studies, more than twice as many Pm were found to move toward roots already infected with this nematode than towards uninfected roots. This observation may have considerable relevance to the behavior of the nematodes in soil. The nematodes move in soil at random seeking an adequate root upon which to feed. Although uninfected host roots may attract the nematodes to some extent, a nematode-infected root will signal its existence as a nutrient source, attracting other nematodes with a much stronger intensity. This may explain the common observation, that nematodes tend to swarm around certain roots while many others are left intact.

A key finding made in the Israeli studies was that root exudates from nematode-infected plants were affecting sporulation of Vd at the root tip. Exudates from nematode-infected plants seem to contain a stable material that can trigger the sporulation of Vd in a root-free system. Further investigations in this area will focus on extraction and biological separation of these exudates to identify the specific material(s) responsible for this phenomenon. Our working hypothesis, however, is that asparagine is connected to sporulation of Vd *in vitro* and, from our experience, asparagine stimulates Verticillium sporulation *in situ*. Whatever the factor that is responsible for the stimulation, the significance of this observation is clear. Microsclerotia of Vd are dispersed widely in soil and at rather low populations. If exudates from nematode-infected roots can trigger more rapid

or extensive germination of Vd propagules in soil, root infection by Vd should be enhanced, hence an explanation for at least one portion of the interaction.

The histological studies conducted in Ohio tend to support the hypothesis that *P. penetrans* interacts with the host in a manner that promotes root and stem colonization of the vascular tissues by *V. dahliae*, even though root tip infection may be similar regardless of the nematode species present in the soil and roots. In general, judging from the low number of root tips infected (less than one per meter of root and less than 10% of all root tips), the probability of any one root tip becoming infected by Vd may be very low, given the inoculum density used in this experiment. This may not differ with the *Pratylenchus* sp. present, but may be greater than roots growing in soil infested with *V. dahliae* alone, which correlates nicely with the Israeli observations about enhanced germination of Vd in association with nematode-infected roots. It appears, however, the probability of subsequent colonization of the root may increase when *P. penetrans* is present, but not when *P. crenatus* or no nematodes are present. Finally, once vascular colonization by Vd is established in the roots, the probability that colonization of the stem occurs becomes very high. At 3 and 5 wk after planting, approximately 0.05% and 0.12%, respectively, of the root was colonized for plants growing in soil infested with both Vd and Pp, while an average of 58% of the plants were colonized in basal stem segments after 5 wk. Results of these studies also indicate that any interaction that might occur on potato between *V. dahliae* and *Pratylenchus* spp. takes place early in the life of the plant, and by the time the stem is colonized, any possible interaction has already occurred.

From these observations, other hypotheses can be stated and tested. It appears that the absence of a host response in the root tip and/or the timing of the host response may be primary factors allowing entry of Vd into the vascular system, and that this may be mediated by Pp. The spatial and temporal aspects of this interaction among the pathogen, nematode, and host are not yet known and further speculation about the processes involved would be inappropriate. Further experimentation using individual roots in a soil environment, and dealing specifically with root tips and the time frame of infection and host response may provide more detail to the above observations and increase our understanding of potato early dying.

One of the reasons microsclerotial inoculum appears to be inefficient in causing root tip infections may be that root growth or elongation is rapid. Larger roots grew at a rate of approximately 1.5 cm/day when directly measured against an observation face in a root box. A growing root tip may pass by a microsclerotium so fast that by the time germination occurs, stimulated by root exudates from the root tip area (26), and hyphae grow toward the root, the susceptible portion of the root (root tip and area of elongation) may be beyond the microsclerotium, especially if the rhizosphere width is small and the microsclerotium needs to be very close to the root to germinate and cause infection (12). Infection may occur more frequently on slower growing roots or a root tip may pass by a microsclerotium that already is in the process of germination, having been stimulated by another root tip that had passed by previously. Since microsclerotia can germinate more than once (6), the second, third, or fourth germination may proceed rapidly and in time for

infection to occur before the susceptible area of the root tip moves out of range. Any factor, such as nematode infection, which stimulates root tip production (i.e. laterals) may increase the probability of a root tip encountering a previously germinated microsclerotium, which then would have a better chance to infect the root. Also, as a root system ages and becomes more profuse, the rate of root tip elongation may decrease. Plant or soil resources may not be able to sustain rapid root growth, thus allowing germination and infection to proceed in a timely manner with an increase in inoculum efficiency.

The studies reported herein certainly do not provide any definitive answers to the mechanisms of interaction between *Verticillium dahliae* and root-lesion nematodes. However, several key findings point to a scenario of multiple factors that affect this interrelationship. It appears that co-infection of roots with nematodes may stimulate increased germination of Vd propagules in the rhizosphere. In light of the low percentage of root infections found in this study that can still result in high amounts of stem infection, any factor that enhances the infection efficiency of Vd propagules in soil may alter the course of disease. Once infection has occurred, it appears that nematode infection may also affect the development of the fungus within an infected root tip. Host resistance factors may in some way be mediated by nematode infection resulting in an increased likelihood of successful vascular infection.

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EVALUATION OF RESEARCH ACHIEVEMENTS

The original objectives of this proposal were to study the effects of root infection by *Pratylenchus* species on activity of *Verticillium* in the rhizosphere and at the root surface, the behavior of both pathogens during interactive colonization, and the effects of *Pratylenchus* infection on host hormonal balance and any relationship this might have on disease development. We have made considerable progress on attaining these objectives and thus have a much better understanding of this complex interaction than when we began.

Studies in both the field and under controlled conditions confirmed the importance of root lesion nematodes, both *Pratylenchus penetrans* and *P. mediterraneus*, in the development of potato early dying. Exudates from nematode-infected roots, possibly containing asparagine, were shown to affect sporulation by *Verticillium dahliae* (Vd) at the root tip, and it appears that co-infection of roots with nematodes may stimulate increased germination of Vd propagules in the rhizosphere. In light of the low percentage of root infections found in this study that can still result in high amounts of stem infection, any factor that enhances the infection efficiency of Vd propagules in soil obviously may alter the course of disease. Once infection has occurred, it appears that nematode infection may also affect the development of Vd within an infected root tip. The most important overall finding of this study, however, is that any interaction on potato between Vd and *Pratylenchus* spp. takes place early in the life of the plant, prior to the time Vd has colonized the base of the stem.

These studies, though basic in nature, have a very practical application to agriculture in both the USA and Israel. Wilt diseases are a serious factor limiting production in both countries of not only potatoes, but many other crops. Nematodes are a complicating factor that results in increased disease losses in several wilt diseases. At present, both soilborne wilt fungi and nematodes are controlled either by soil fumigation or by the development of host-resistant cultivars. Soil fumigants are increasingly under attack because of environmental problems and thus efforts must be redoubled to develop viable resistant cultivars. Unfortunately host resistance factors may in some way be mediated by nematode infection, resulting in an increased likelihood of successful infection by wilt fungi. In order to develop an effective management strategy for wilt diseases based on the use of resistant cultivars, our understanding of the role of nematodes in the disease complex must be improved. In addition, a better understanding of the processes by which wilt disease fungi become established may lead to new approaches to their control that are not yet conceived.

COOPERATION BETWEEN OHIO AND ISRAELI SCIENTISTS

This work originated as a result of a trip by Drs. Rowe and Riedel to Israel in 1987 to visit with Drs. Nachmias and Orion at the Volcani Center and the Gilat Experiment Station. After the grant period began, Dr. Orion traveled to Ohio in May, 1990, to visit The Ohio State University and the Ohio Agricultural Research and Development Center in order to discuss plans and further develop common research approaches. In June, 1990, Ms. Yael Rotman, an M.Sc. student on the project with Dr. Orion, spent three weeks in Ohio with Drs. Rowe and Riedel to learn techniques in their labs and to teach some techniques in use in Dr. Orion's lab. Dr. Orion again visited Ohio in July, 1991, and spent several days in both labs there and considerable time interacting with Dr. John Bowers, postdoctoral research associate with Drs. Rowe and Riedel. Both these visits by Dr. Orion were useful to discuss mutual progress towards our research objectives. In November, 1992, Dr. Rowe visited Israel and spent several days in the laboratories of Drs. Nachmias and Orion to discuss research progress and begin to develop an outline for this final report. Although research in both countries was carried out quite independently, there was regular communication and discussion of mutual progress. An additional result of this international cooperation is the sponsorship of an International Verticillium Conference in June of 1993 in Israel. Both Drs. Nachmias and Rowe are on the organizing team.

PUBLICATIONS TO DATE THAT HAVE RESULTED FROM RESEARCH SUPPORTED BY BARD GRANT US-1501-88R

Bowers, J.H., Nameth, S.T., Riedel, R.M., and Rowe R.C. 1991. Effect of *Pratylenchus penetrans* and *P. crenatus* on infection and colonization of potato by *Verticillium dahliae*. Phytopathology 81:1165 (Abst).

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