AN ABSTRACT OF THE THESIS OF

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(Name) (Degree)

in Botany and Plant Pathology presented on August 15, 1969
(Major) (Date)

Title: THE INTERACTIONS OF PRATYLENCHUS PENETRANS WITH HOST PLANTS, LONGIDORUS ELONGATUS AND VERTICILLIUM DAHLIAE

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The root-lesion nematode, Pratylenchus penetrans (Cobb 1917) Filipjev and Shuurmans Stekhoven, 1941, is widely distributed throughout Oregon and other temperate zones of the world. Economically it is probably the most important plant parasitic nematode in Oregon because of its abundance, wide host range of economic plants and interactions with other plant pathogens in disease complexes.

A host range study of this nematode included many hosts which fall into one of the four categories: vegetables, field crops, ornamentals and weeds. Most of the plants included are commercially grown in Oregon or are troublesome weeds. The hosts were given a susceptibility rating. Most plants were found to be susceptible. Some of the more susceptible hosts included the clovers, pea, radish, sunflower and the weeds lambsquarter, pigweed and wild carrot. Some plants found to be only slightly susceptible were bean, pepper,

sugar beet, many grasses and wild oats. The African marigold was found to be not susceptible.

New types of pear rootstocks are being actively sought to provide dwarfness and disease resistance. A test of the susceptibility to P. penetrans of several Pyrus spp. was conducted for additional information. Pyrus ussuriensis Maxim. was found to be very susceptible and P. Calleryana Dcn., a pear decline resistant rootstock, and P. syriaca Boiss., a dwarf rootstock, to be slightly susceptible. Other species were intermediate.

An interaction between two plant nematodes, P. penetrans, an endoparasite, and Longidorus elongatus (de Mann, 1876) Thorne and Swanger, 1936, an ectoparasite, was investigated using peppermint as the common host. Results indicated that when the population of either one of the nematodes was high the level of the other was significantly lower. This is probably due to competition between the two nematodes for food and space.

The association between P. penetrans and the fungus Verticillium dahliae Kleb. was studied. The two pathogens were grown in combination and separately on peppermint in pots in the greenhouse. Upon harvest the rhizosphere of the peppermint roots was assayed for propagules of V. dahliae. Results indicated that when the nematode was present there was a significant increase in the number of fungal propagules in the rhizosphere. This increase may constitute the necessary increase in inoculum potential of the fungus to cause the increased incidence of wilt which has been observed by several authors when the fungus and nematode are found together.

There are many facets to the pathogenicity of *P. penetrans*. Direct damage to the host as a result of feeding on host tissues is but one facet. Control of this type of injury may be achieved by utilizing host range knowledge in selection of cover and rotation crops and in weed control. Interaction of this nematode with other plant pathogens greatly increases its importance as a pathogen. Adequate control of soil fungal diseases may be realized when the influence of soil nematodes on such diseases is better understood.

The Interactions of *Pratylenchus penetrans* With Host Plants, *Longidorus elongatus* and *Verticillium dahliae*

by

John McKay Yorston

A THESIS

submitted to

Oregon State University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

June 1970

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Date thesis is presented August 15, 1969

Typed by Muriel Davis for John McKay Yorston

ACKNOWLEDGMENT

The author wishes to express sincere gratitude to Dr. Harold J. Jensen for his counsel, constant encouragement and patience during the author's stay in this Department and especially throughout the preparation of this manuscript. Thankful appreciation is extended to Drs. T. C. Allen, Jr. and H. Mack for their helpful criticism, and to Mrs. Mary Ann Strand for her statistical assistance.

The author also wishes to express his thankfulness to the Oregon Agricultural Station and to the Department of Botany and Plant Pathology for awarding a research assistantship, and for providing space and facilities, for this research project.

Special thanks to Mrs. Bruce for typing the rough draft.

TABLE OF CONTENTS

LIST OF TABLES

THE INTERACTIONS OF *PRATYLENCHUS PENETRANS* WITH HOST PLANTS, *LONGIDORUS ELONGATUS* AND *VERTICILLIUM DAHLIAE*

INTRODUCTION

Economically the root-lesion nematode, *Pratylenchus penetrans* (Cobb, 1917), Filipjev and Shuurmans Stekhoven, 1941, is probably the most important plant parasitic nematode in Oregon. It has a vast host range including many economic plants and is widely distributed although appearing mainly in cultivated areas. *P. penetrans* is particularly a pest in nurseries. Because of the importance of *P. penetrans* in Oregon this study was organized to investigate the host range and environmental and ecological relationships of the nematode.

Knowledge of the host range of *P. penetrans* is important when rotation and cover crops are being selected and when a new site for the development of a commercial enterprise, such as a nursery, is being considered. It is important to select plants which have the lowest susceptibility rating so that nematode populations will not build up to damaging levels. Many plants commonly grown in Oregon were tested for their degree of susceptibility to the root-lesion nematode. From this information recommendations can be made for crop selection.

There also is considerable interest in new types of rootstocks for commercial production of pear. Dwarfness and disease resistance are of particular interest. A sizable collection of Pyrus spp. is maintained at Oregon State University. This study was undertaken to obtain further information about the rootstocks with respect to their susceptibility to the root-lesion nematode. Such information will be useful at the time of selection of a rootstock, especially in areas where the nematode is present.

One of the more important areas of study in nematology today is the complex interrelationship between nematodes and other plant pathogens including bacteria, fungi, viruses and other nematodes. Two such relationships are studied.

Almost no research has been done in the area of the interrelationships of two plant parasitic nematodes. During routine laboratory work it was noted that when greenhouse material became contaminated with a foreign nematode the resulting population level was influenced. For these two reasons studies were conducted to determine the influence of one nematode population on another.

The second relationship investigated was that between a plant parasitic nematode and a fungus. This relationship is the best understood and is possibly the most important economically. Both organisms may be destructive pathogens when acting alone; however their combined pathological potential is often far greater than the sum of their individual effects. This study was designed to investigate the relationship between the root-lesion nematode and a wilt producing fungus. The main objective of the experiment was to determine if the nematode influences the inoculum potential of the fungus. This information will help explain how the presence of the nematode increases the severity of the disease.

LITERATURE REVIEW

Host Range

There are many publications listing plants as hosts of *Pratylenchus penetrans*. Oostenbrink, s'Jacob and Kuiper (1957) have published the most extensive list of host plants. This paper listed 182 plants including deciduous trees, ornamentals, vegetables and forage crops. Jensen (1953) published a list of susceptible plants tested in the greenhouse. These plants were mostly grasses and legumes. Townshend and Davidson (1960) published a list of 55 species of weed hosts. These three papers alone list 232 plant species belonging to 126 general and 51 families. In addition, Sutherland (1965), reported several conifers in Quebec forest nurseries as being hosts. These publications and many others not cited indicate that nearly all plants which have been tested are hosts of *P. penetrans*.

Cover Crops and Rotations

Several workers have made recommendations of crops to be used in a rotation or as cover crops. The main objective is to provide a cultural control of nematode pests. Oostenbrink (1956) investigated rotations in Holland nurseries. He found the population level of *P. penetrans* to be highest after cereals, less after potato, *Laburnum* and *Rosa canina* and least after beet and woody crops other than the nursery crop being grown. Thus it was deduced that attacks on potatoes will increase in a rotation following cereals such as rye and oats and should decrease if potato follows beet in the rotation. For the cultivation of woody nursery plants, no rotation has been devised which is safer than continuous growth of the nursery crop. At a later date Oostenbrink (1961) made further recommendations for nursery crop rotations. He advised avoiding cultivation of potato, oats, rye and red clover in nurseries infested with *P. penetrans* and growing sugar beets or marigolds prior to potato or red clover on heavily infested arable land. He recommended that red clover be omitted on infested soil.

Slootweg (1956) first reported the usefulness of African marigold (*Tagetes erecta* L.) in reducing the population of *P. penetrans*. He discovered by accident that root rot of narcissi caused by *P. penetrans* and a secondary invasion by the fungus *Cylindrocarpon radicola* Wr. was greatly reduced by prior cultivation of marigold. The improvement, however, was less than that obtained from soil fumigation with Shell DD. He showed that *T. erecta* reduced the population of *P. penetrans* to one tenth the original level after other crops commonly grown in Holland nurseries. Also Slootweg suggested that *T. erecta* could be used instead of Shell DD where it was too expensive or impractical to fumigate because of adverse soil conditions.

Oostenbrink (1957) found very high populations of P. penetrans developed on red clover which reversed the otherwise useful effect of the crop as green manure. He stated that on the other hand T. erecta appeared to suppress P. penetrans and was therefore useful as a rotation crop. T. erecta also was considered a good green manure crop.

Two Dutch workers, Uhlenbroek and Bijloo (1958), isolated a nematicidal agent from Tagetes roots. Several highly nematicidal polythienyls were isolated, one of which was identified as α-terthienyl. In vitro, the compound was nematicidal against several plant pathogenic nematodes besides P. penetrans.

Hoesta and Oostenbrink (1962) described the influence of cover crops under orchard trees on the nematode population in and around the tree roots. Cover crops of Tagetes patula L., red clover, apple seedlings, and fallow were used in apple plantings on infested soil. The P. penetrans population was high on red clover, intermediate on fallow and apple seedlings and low on T. patula plots. Orchards undersown with arable crops such as potato, cereals, red clover or grass and clover mixed may support much denser soil populations than corresponding clean cultivated areas. This is especially true soon after crops have been lifted or plowed under.

McBeth and Taylor (1944) significantly increased the growth of peach trees by planting root-knot resistant cover crops. These resistant cover crops reduced the root-knot nematode populations to a level where less economic damage was done to the peach trees. McDonald and Mai (1963) searched for such resistant cover crops for sour cherry orchards in an attempt to reduce damage by the root-lesion nematode. They found Sudan grass (Sorghum vulgare var. sudanese (Piper) Hitchc.) to be a poor nematode host. Of other crops tested, perennial rye grass (Lolium perenne L.) also had a fairly low population in its roots. Several other crops including sweet clover, Japanese millet, hairy vetch, rape, oats and soybean support high populations of root-lesion nematodes. These plants should not be used as a cover crop.

Susceptibility of Pear Root Stocks (Pyrus spp.) to P. penetrans

With the advent of pear decline, a virus disease transmitted by pear psylla, there has been considerable interest in species of Pyrus for use as pear rootstocks. The trend towards dwarf rootstocks has also created interest in Pyrus syriaca, Boiss., P. Fauriei, Scheid. and P. pashia, Ham. According to Westwood and Lombard (1966) trees with a P. ussuriensis, Maxim. rootstock are quite susceptible to pear decline. When P. Calleryana, Dcn. is used the incidence of pear decline is much less severe. P. Calleryana is also much more tolerant to wet soils than P.

ussuriensis. Because of these advantages there is considerable interest in *P. Calleryana* as a commercial rootstock.

Oostenbrink (1954) mentioned pear as one of several nursery rootstocks which are susceptible to *P. penetrans*. Decker (1960) also listed pear as a host. Jensen (1961) found *P. penetrans* to be a common pest of pear seedlings. French *et al*. (1964) investigated the possibility of *Pratylenchus spp*. being involved in pear decline. They found *P. penetrans* associated with pear rootstocks but found no correlation between this association and the incidence of pear decline. None of the above references gave the name of the species of pear rootstock involved.

Longidorus, Pratylenchus Interaction

Graham, Ford and Currin (1964) observed possible competition between *Meloidogyne incognita acrita* Chitwood & Oteifa, 1952 and *Pratylenchus spp*. The two nematodes were found interspersed in the same field plots. Where the population of the root-lesion nematode (*Pratylenchus spp.*) was found to be high, the root-knot (*Meloidogyne*) index was found to be low and erratic. The root-lesion nematode was assumed to be dominant.

Malek and Jenkins (1964) found competition among three different nematode species, *Trichodorus christiei* Allen 1957, *Criconemoides curvatum* Raski, 1952 and *Meloidogyne hapla*

Chitwood, 1949. These two nematodes cohabited the root zone of hairy vetch (*Vicia villosa* Roth). Comparisons were made of population levels and of the amount of damage done to the plants when the nematodes were in various combinations. Several relationships were found.

The reduction in top weight was greatest when *T. christiei* was included in the complex. The most severe reduction in top growth occurred in the combination of *T. christiei* and *M. hapla*. The numbers of *T. christiei* were lowest in combination with *M. hapla*. The latter produces root galls thus limiting the initial feeding sites for *T. christiei*. The final population of *C. curvatum* was lowest when all three species were together. The combination of *T. christiei* had more effect in suppressing *C. curvatum* than did the combination with *M. hapla*. *T. christiei*, being a vagrant feeder and increasing in numbers rapidly, evidently was more efficient than *M. hapla* in reducing the number of feeding sites for *C. curvatum* and actively crowded out the latter. *T. christiei* was not markedly affected by the presence of *C. curvatum* although the reverse is not true. The latter evidently cannot efficiently compete with the former on the roots of hairy vetch.

In some cases mutual feeding affected the host more severely than an individual species alone. The combined effect of *T. christiei* and *M. hapla* on the growth of hairy vetch was more than additive and may constitute synergism.

Pratylenchus - Verticillium Complex

Atkinson (1892) reported the first disease complex involving nematodes and fungi. He reported that root-knot nematode increased the severity of *Fusarium* wilt in cotton. The first report of a fungus-nematode complex involving *Pratylenchus sp.* was by Hastings and Bosher (1938). They showed that the nematode alone reduced the growth of potato, carrot, red clover, tomato, spinach and violet by 50-75 percent. The interacting fungus, *Cylindrocarpon radicola* Wr., reduced growth by 6 to 11 percent. When the fungus and nematode occurred together the reduction in growth was usually greater than the sum of reductions of both organisms alone.

Root-lesion nematodes, *Pratylenchus spp.*, have been associated by many authors with *Verticillium* wilt of several crops. Abu-Gharbieh, Varney and Jenkins (1962) inoculated soil in which strawberry varieties resistant, moderately resistant and highly susceptible to *Verticillium* wilt were grown with *Pratylenchus penetrans* alone, *Verticillium albo-atrum* Reinke & Berth. alone and combinations of the fungus and nematode. The highly susceptible plants inoculated with both pathogens developed wilt symptoms earlier and more severely than with *Verticillium* alone. There was no apparent interaction between the pathogens on the moderately resistant and resistant varieties. Therefore the author deduced that wilt resistance appeared to be physiological rather than mechanical.

Rich and Miller (1964) reported that various fungicides, particularly Terrachlor applied to the soil, increased the incidence of Verticillium wilt of strawberries in the field. The increase in Verticillium wilt was correlated with an increase in the Pratylenchus penetrans population.

Faulkner and Skotland (1965) showed a striking relationship between Verticillium dahliae and Pratylenchus minyus Sher & Allen on peppermint. Greenhouse plants were inoculated with Verticillium and the nematode, alone and in combination. Presence of nematodes increased the number of plants expressing wilt symptoms and increased the severity of the symptoms. The incubation period of the wilt was reduced by two to three weeks when nematodes were present. Dry weight of the plants was reduced by approximately 21 percent by either pathogen alone and by 68 percent when the two were together. An interesting observation was that reproduction of the nematode approximately doubled in the presence of Verticillium.

Olthof and Reyes (1969) used pepper as a host plant for an experiment using Pratylenchus penetrans and Verticillium dahliae. The two pathogens were used alone and in combination on the host. They found all three treatments significantly reduced heights of the plants; however, the damage done by the nematode and fungus was found to be additive.

Bergeson (1963) reports that when P. penetrans-infected peppermint plants were inoculated with V. albo-atrum, the diagnostic symptoms of Verticillium wilt appeared approximately two weeks earlier than when the fungus was present alone. This did not occur, however, with simultaneous inoculations of fungus and nematodes.

In growth room experiments, Mountain and McKeen (1962) grew tomato seedlings in steam sterilized soil infested with V. dahliae alone, with P. penetrans alone and with both pathogens together. After 40 days, plants in the soil inoculated with the fungus and the nematode together showed consistently higher incidence of wilt than did plants in the soil inoculated with the fungus alone. Miller and Edington (1962) reported a similar relationship in tomato. They showed that when soil amendments of sawdust or chopped paper were added to soil infested with P. penetrans and inoculated ten days later with microsclerotia of V. albo-atrum, the nematode population decreased and consequently there was a reduction in the severity of wilt in tomatoes.

Edmunds and Mai (1966) found populations of P. penetrans to be higher in roots of eggplant and tomato, which were infected with V. dahliae, than in roots without the fungus. The same was not true, however, for pepper. P. minyus and P. penetrans also increased to a greater extent in roots of peppermint (Mentha piperita) infected with V. dahliae and V. albo-atrum, respectively, than in roots not infected with these fungi.

Morsink (1967) reported wilt ratings significantly higher in the combined treatment of P. penetrans and V. albo-atrum on potato than at corresponding treatments with only the fungus. The wilt ratings were greatest in the highest fungus, nematode concentrations. More nematodes were found in the soil at all fungus levels in combination treatments than in treatments with only the nematodes and the most abundant numbers were found in soil with the highest level of fungus inoculum.

Mountain and McKeen (1960) discovered that soil fumigation with nematicides exerted a temporary but appreciable reduction in the incidence of Verticillium wilt of eggplant which was grown in soil infested with P. penetrans. Greenhouse experiments were set up with both pathogens alone and in combination. Varying levels of Verticillium inoculum were used. The nematode had no apparent effect on the incidence of wilt at high soil inoculum levels of Verticillium. There was a relative increase in wilt, however, in the presence of the nematode as the amount of fungus inoculum was progressively reduced. The evidence showed that wilt increase was related to the number of nematodes involved. The nematodes alone had no adverse effect on the root condition of the eggplant.

McKeen and Mountain (1960) found that in the absence of V.

albo-atrum, P. penetrans had no significant effect on eggplant seedlings grown in pots even at levels as high as 4,000 nematodes per pot. The fungus alone was pathogenic but the addition of nematodes greatly enhanced the effectiveness of the fungus. The most interesting observation of this experiment was the increase in the number of nematodes in the roots of plants infected with the fungus over the number in the control treatment of the nematode alone. These observed relationships may constitute a synergistic relationship between the two pathogens. Since the ratio of juvenile to adult nematodes was usually greater when the fungus was present, it is possible that the fungus rendered the root substrate more suitable for nematode reproduction.

The effect of the nematode increasing the incidence of the fungal wilt may be directly mechanical. The nematode wound may be providing a direct entry for the fungus and may thus be facilitating the progress of the fungus towards the vascular system of the root.

Dwinnell (1967) found that any nutritional regime which altered the susceptibility of maples or elms to Verticillium dahliae also affected their adequacy as hosts of P. penetrans. As wilt severity increased in elm and maple seedlings, the relative nematode population in their roots increased and decreased respectively.

It is apparent that nematodes are directly affected by changes in the physiology of the host plant which are induced by interactions of the fungus and host under varying conditions of host nutrition.

HOST RANGE

Methods

Many plants belonging to one of the four categories, vegetables, field crops, ornamentals and weeds, were grown in a greenhouse to test the host range of the root-lesion nematode Pratylenchus penetrans. Number 2-1/2 cans, which have a volume of one quart, were filled with soil containing the nematodes. Two trials were carried out, the inoculum level in the first trial was 750 P. penetrans per quart of soil, and in second trial was 1, 600 P. penetrans. Four replications were used for each plant species tested. Several seeds were planted in each can and the seedlings were thinned to an appropriate number depending upon the growth habit of the plant.

After six to eight weeks the plants were harvested by soaking them in a tub of water and freeing the roots from the soil mass. The roots were washed clean under a water jet, dried with a paper towel and weighed to the nearest tenth of a gram. The clean roots were cut into one-quarter inch lengths and placed into a Waring blender containing 100 ml. of water. This mixture was blended at low speed for ten seconds, placed in a Baermann funnel for four days, drained into a large test tube, and examined with a dissecting microscope for nematodes. The number of P. penetrans per gram of roots was calculated.

Results

The results of the host range given in Tables 1-6 show that the host range of *P. penetrans* was very diverse, including all plants tested except Tall African marigold, *Tagetes erecta* L. Because the number of nematodes found per gram of roots varied considerably, the plants were given a sensitivity rating. A separate sensitivity rating was constructed for each of the two trials.

Several comparisons were made between the two trials to show that the relative level of nematode infestation of host roots was lower in trial 1 (initial nematode population 1, 600 per quart). Comparisons of the two trials indicate: 1) expected increase in trial 2 (radish and cauliflower), 2) greater than expected increase in trial 2 (Alsike clover), and 3) unexpected drop in trial 2 (Alaska pea).

In the case of radish and cauliflower the same susceptibility rating of very susceptible (+++) was given in both trials. Alsike clover in trial 2 (Table 5) had a higher susceptibility rating of quite susceptible (++). This compared to a moderately susceptible rating (+) for the same plant in trial 1 (Table 4). This is explained by the fact that Alsike clover in trial 2 was on the borderline between the two arbitrary categories of moderately susceptible (+) and quite susceptible (++).

It is difficult to explain why the nematode population in the

Alaska variety of garden pea was higher in trial 1 than in trial 2. It may be partly explained on the basis of the difference in total root weight of plants in the two trials. In trial 1 the average root weight of four replications was 1.2 grams and in trial 2 the average root weight was 4.9 grams. The total number of nematodes present in roots of peas in trial 1 was 212 and in trial 2 was 294. Since there is a limited number of nematodes in a can of soil, and when the root system of the plant is extensive, the potential number of nematodes attracted to and entering the plant roots would be less per gram of roots than if the root system of the plant were less extensive. This concept also may explain why the grasses have such low susceptibility ratings as they have extensive, fibrous root systems.

Discussion

The damage or injury level of the nematode will vary considerably from one plant species to another species. Injury takes forms other than just mechanical root injury which may result in reduced yields. An example of mechanical injury is given by Goheen and Smith (1956). They found that as a result of Pratylenchus penetrans feeding upon strawberry roots the plants were dwarfed and the yields reduced.

Some aspects of the mechanism of mechanical injury have been worked out by Mountain and Patrick (1959). They induced

Explanation of Susceptibility Rating Symbols

Trial 1. (initial population *P. penetrans* 750 per quart of soil)

	Symbol	No. of *P. penetrans* per gram of roots
not susceptible	-	0
slightly susceptible	0	< 1-2
moderately susceptible	+	3-10
quite susceptible	++	11-25
very susceptible	+++	25 >

Trial 2. (initial population *P. penetrans* 1, 600 per quart of soil)

	Symbol	No. of *P. penetrans* per gram of roots
not susceptible	-	0
slightly susceptible	0	< 1-3
moderately susceptible	+	4-24
quite susceptible	++	25-50
very susceptible	+++	50 >

Table 1. Susceptibility of vegetables to Pratylenchus penetrans - Trial #1

Family	Plant	Variety	Binomial Name	Nematodes per gram of roots	Suscepti-bility rating
Chenopodiaceae	Beet	Blood	Beta vulgaris L.	5	+
Compositae	Lettuce	Great Lake	Latuca sativa L.	22	++
Cruciferae	Broccoli	Waltham 29	Brassica oleraceae L. var. botrytis L.	0.3	0
	Brussels Sprouts	Catskill	Brassica oleraceae var. gemmifera D.C.	1	0
	Cabbage	Danish Baldhead	Brassica oleraceae var. capitata L.	6	+
	Cabbage	Mammoth Red Rock	Brassica oleraceae var. capitata L.	2	0
	Cauliflower	Snowball Y	Brassica oleraceae L. var. botrytis L.	15	++
	Radish	French Breakfast	Raphanus sativus L.	30	+++
	Rutabaga	American Purple Top	Brassica campestris var. napobrassica (L.) D.C.	1	0
	Turnip	Shogoin	Brassica rapa L.	11	++
Cucurbitaceae	Cucumber	Boston Pickling	Cucumis sativus L.	13	++
	Melon	Chilean	Cucumis melo L.	6	+
Gramineae	Corn	Sweet	Zea mays var. saccharata (Sturtev) Bailey	5	+
Leguminoseae	Bean	FM-1	Phaseolus vulgaris L.	2	0
		FM-1K		2	0
		FM-65		5	+
		G-50		5	+
		OSU 949-1864-2		2	0
		Pure Gold		2	0
	Pea	Alaska	Pisum sativum	65	+++
Lilliaceae	Onion	Bermuda	Allium cepa L.	42	++
Solanaceae	Pepper	Yola Wonder L.	Capsicum frutescens L.	0.3	0
	Tomato	Bonny Best	Lycopersicon esculentum Mill.	5	+
Umbelliferae	Carrot	Royal Chantenay	Daucus carota L. var. sativa D.C.	5	+
	Dill		Anethum graveolens L.	18	++

Table 2. Susceptibility of vegetables to Pratylenchus penetrans - Trial #2

Family	Plant	Variety	Binomial Name	Nematodes per gram of roots	Susceptibility rating
Chenopodiaceae	Spinach	Zealand	*Spinacia oleracea* L.	44	++
Crucifereae	Cauliflower	Snowball Y	*Brassica oleraceae* L. var. *botrytis* L.	71	+++
	Radish	Early Scarlet White Top	*Raphanus sativus* L.	70	+++
Cucurbitaceae	Cucumber	Boston Pickling	*Cucumis sativus* L.	26	++
Leguminosae	Bean	Beca	*Phaseolus vulgaris* L.	3	0
	Bean	Pinto	*Phaseolus vulgaris* L.	1	0
	Garbanzo Bean		*Cicer arietinum* L.	6	+
	Lima Bean	Baby	*Phaseolus lunatus* L.	1	0
	Pea	Alaska	*Pisum sativum* L.	15	+
Solanaceae	Eggplant		*Solanum melongena* L.	5	+
	Pepper		*Capsicum frutescens* L.	0.3	0

Table 3. Susceptibility of ornamentals to Pratylenchus penetrans - Trial #2

Family	Plant	Variety	Binomial Name	Nematodes per gram of roots	Susceptibility rating
Compositae	Marigold	Tall African	*Tagetes erecta* L.	0	-
Euphorbiaceae	Castor Bean		*Ricinus communis* L.	19	+
Leguminosae	Sweet Pea		*Lathyrus odoratus* L.	68	+++
Scrophulariaceae	Snap Dragon		*Antirrhinum majus* L.	17	+
Solanaceae	Petunia	Mixed Balcony	*Petunia hybrida* Vilm.	92	+++

Table 4. Susceptibility of field crops to Pratylenchus penetrans - Trial #1

Family	Plant	Variety	Binomial Name	Nematodes per gram roots	Susceptibility rating
Chenopodiaceae	Sugar beet		Beta vulgaris L.	2	0
Graminae	Oats	Victory	Avena sativa L.	8	+
	Rye	Abruzzi	Secale cereale L.	3	+
	Wheat	Redmond	Triticum aestivum L.	0.5	0
Leguminosae	Clover	Alsike	Trifolium hybridum L.	4	+
	Vetch	Hairy	Vicia villosa Roth	2	0
Solonaceae	Potato		Solanum tuberosum L.	3	+

Table 5. Susceptibility of field crops to Pratylenchus penetrans - Trial #2

Family	Plant	Variety	Binomial Name	Nematodes per gram roots	Susceptibility rating
Compositae	Sunflower		Helianthus annuus L.	241	+++
Gramineae	Annual Blue Grass		Poa Annua	1	0
	Annual Rye Grass		Lolium temulentum L.	1	0
	Chewing's Fescue		Festuca rubra L. var. commutata Gaud.	0.2	0
	Creeping Bent Grass		Agrostis palustris Huds.	0.4	0
	Orchard Grass		Dactylis glomerata L.	2	0
	Perennial Rye Grass		Lolium perenne L.	1	0
	Sudan Grass		Sorghum vulgare var. Sudanese (Piper) Hitchc.	1	0
	Smooth Brome Grass		Bromus inermis Leyss.	4	+
	Tall Oat Grass		Arrhenatherum elatius (L.) Presl.	2	0
	Timothy		Phleum pratense L.	6	+
Libiatae	Peppermint		Mentha piperita L.	3	0
Leguminosae	Alfalfa		Medicago sativa L.	36	++
	Clover	Alsike	Trifolium hybridum L.	26	++
	Clover	Crimson	Trifolium incarnatum L.	2	0
	Clover	Sweet	Melilotus alba Desr.	25	++
	Clover	White	Trifolium repens L.	8	+
	Vetch	Common	Vicia sativa L.	4	+
	Vetch	Hairy	Vicia villosa Roth	5	+

Table 6. Susceptibility of weeds to <u>Pratylenchus penetrans</u>

Family	Plant	Variety	Binomial Name	Nematodes per gram roots	Suscepti-bility rating
		Trial #1			
Compositae	False dandelion		*Hypochoeris radicata* L.	1	0
		Trial #2			
Amaranthaceae	Pigweed		*Amaranthus retroflexus* L.	76	+++
Chenopodiaceae	Lambsquarter		*Chenopodium album* L.	105	+++
Compositae	Bull thistle		*Cirsium vulgare* (Savi) Tenore	154	+++
	Dandelion	Common	*Taraxacum officinale* L.	3	0
	Dog Fennel		*Eupatorium capillifolium* (Lam.) Small.	20	+
Cruciferae	Mustard		*Brassica kaber* (D.C.) L. C. Wheeler	9	+
Graminae	Wild Oats		*Avena fatua* L.	1	0
Plantaginaceae	Plantain		*Plantago major* L.	5	0
Umbelliferae	Wild Carrot		*Daucus carota* L.	67	+++

necrotic lesions on the roots of peach seedlings under aseptic conditions. Within nine hours after the nematode came in contact with the surface of the peach root the nematode was partly buried in the root tissue. By this time the discolored area about the entry point had developed into a distinctly visible tan-colored lesion, the cells of which were necrotic with shrunken, granular cytoplasm. Cells appreciably in advance of the point of nematode entry showed these effects. This suggests there is a toxic substance in advance of the invading nematode.

Patrick (1955) did some work on the chemical reaction involved in lesion formation. He showed that hydrolysis of the cyanophoric glucoside, amygdalin, present in peach roots, resulted in the release of benzaldehyde and hydrogen cyanide in phytotoxic amounts. The nematode, P. penetrans, was found to release substances, likely enzymes, in vitro which hydrolyzed amygdalin. The conclusion reached was that lesion formation in peach roots results from the release of phytotoxic substances through the hydrolysis of amygdalin by the nematode.

The degree of injury depends upon the use of the plant. An ornamental propagative stock for export may be essentially worthless if infected with nematodes although little mechanical damage is done to the plant. Growers may be reluctant to buy stock which is infected for fear of contaminating their soil.

Injury with respect to cover and rotation crops and weeds must be considered from a different point of view. Many of the plants tested in this host range study belong to one or more of these categories. With this type of plant it is the amount of nematode population increase or decrease that is important. The welfare of the crop following, in the case of a rotation crop, or a crop being grown in conjunction, in the case of a cover crop, will depend upon the level of nematodes in the soil. If a large nematode population is harbored in the roots of a rotation or cover crop the soil nematode count may reach a level potentially dangerous to the subsequent crop.

Weeds also may assist the development of a high nematode soil count as is evidenced by the fact that several of the weeds tested had ratings of very susceptible. This fact serves as another reason for maintaining adequate weed control. According to Townshend and Davidson (1960), weeds may act as a reservoir for large numbers of overwintering nematodes, posing a potential threat to any succeeding crop susceptible to this nematode. Plants such as pigweed, lambs quarter, bull thistle and wild carrot, all with very susceptible ratings, are examples of weeds which could provide a nematode reservoir (Table 6).

When considering the nematode population in an orchard the choice of a cover crop is important. A plant is needed which is a poor host for the nematode and which is vigorous enough to be the dominant species in the cover crop. Thus it can crowd out weeds which may be good nematode hosts and negate the beneficial effect of the cover crop. MacDonald and Mai (1963) found Sudan grass (Sorghum vulgare var. sudanese) to be a poor host of P. penetrans and able to grow vigorously enough to become the dominant species of the cover crop.

Hoestra and Oostenbrink (1962) found the cover crop of red clover in an apple orchard to have high numbers of nematodes, apple seedlings and fallow an intermediate number, and Tagetes patula a low number. Results of this experiment indicate that alsike, sweet and white clover also harbor relatively high numbers of nematodes (Table 5). If used as a cover crop, Tagetes erecta also would harbor a low population of P. penetrans. The authors also found that in orchards with permanent pasture cover crops, there are cases of low population density in apple roots which are often associated with high population densities in grass roots. The grass cover crop may be a more efficient host and thus at least temporarily decrease the population in apple roots. Grass may provide a better opportunity for nematode reproduction or nematodes may prefer grass roots to apple roots. Perhaps another nematode biotype is present in the grassland. Most grasses tested in this experiment showed low numbers of nematodes per gram of roots (Table 5). It must be remembered, however, that the total number of nematodes present in the grass root system can be quite high because of their extensive fibrous nature.

Generally the principles involved in cover crops hold true for rotation crops. When a crop which is economically damaged by *P. penetrans* is included in a rotation, care should be taken to avoid crops with high susceptibility ratings. If a new nursery is to be established, the previous cropping history of the field is important for the same reason. Oostenbrink (1961) found that it was advisable to avoid potato, oats, rye and red clover in a rotation on soil with a high *P. penetrans* count. He advised using sugar beet or marigold prior to a potato or red clover crop. The results of this experiment indicate that legumes including alfalfa, clovers (Tables 4 and 5), and garden pea and brassicas (Tables 1 and 2) should be avoided in a rotation. Establishment of a nursery on a soil with such a cropping history should be avoided. Sugar beets tested in this experiment were only slightly infested indicating their usefulness as a rotation crop (Table 4). Garden beans tested were rated as only slightly susceptible and also would be suitable in a rotation.

SUSCEPTIBILITY OF PEAR ROOTSTOCKS (*PYRUS SPP.*) TO *P. PENETRANS*

Methods

Seven different species of the genus *Pyrus* were tested for their susceptibility to a root-lesion nematode, *P. penetrans*. Seedlings two to three inches in height or root cuttings of *Pyrus Calleryana* Dcn. were transplanted into five-inch clay pots containing a quart of soil with 900 *P. penetrans*. Five separate pots of each species were grown for four months in the greenhouse. The same method of removing plants from the pots at harvest time and for removal of nematodes from plant roots was used as described previously for the general host range study. Calculations of the number of nematodes per gram of roots were made and recorded.

Results

The results showed that all species of *Pyrus* tested were susceptible to *P. penetrans* (Table 7). *Pyrus ussuriensis* Maxim. was rated as very susceptible but two species, *P. Calleryana* and *P. syriaca* Boiss. were only slightly susceptible. *P. communis*, which is the most common rootstock did not root in this trial and was therefore not available for comparison with other species.

Table 7. Susceptibility of *Pyrus* *spp.* to *Pratylenchus* *penetrans*

Pear Species	Seedling Number	Average No. of *Pratylenchus* per gram of roots	Susceptibility rating
1. *Pyrus* *amygdaliformis* Vill.	Sardinia seedling	8.0	+
2. *Pyrus* *betulafolia* Bnge.	#3 seedling	19.0	+
3. *Pyrus* *Calleryana* Dcn.		1.5	0
4. *Pyrus* *Fauriei* Scheid.	Max W-6 seedling	23.4	+
5. *Pyrus* *pashia* Ham.	Australia #2 seedling	10.2	+
6. *Pyrus* *syriaca* Boiss.	#4 seedling	3.6	0
7. *Pyrus* *ussuriensis* Maxim.	P.I. - 300	181.6	++

0 - slightly susceptible
+ - moderately susceptible
++ - very susceptible

Discussion

The results indicate that *P. Calleryana* would be useful as a commercial rootstock since it harbored a small nematode population. *P. Calleryana* also is useful because it is a vigorous rootstock, has a low incidence of pear decline and is tolerant to wet soils.

The chemical components of various *Pyrus spp.* have been studied by Challice and Williams (1968). They analyzed leaf phenolics of *P. Calleryana* and revealed that the species was a highly individualistic nember of the genus *Pyrus*. Some of the compounds found in *P. Calleryana* which were not found in other *Pyrus spp.* were alcoholic esters of caffeic, protocatechuic, p-hydroxybenzoic and vanillic acids and callerynin. These C_6-C_1 phenolic acids had not previously been found in *Pyrus*. The relative resistance of *P. Calleryana* to many disease causal agents may be related to its unique phenolic contents.

PRATYLENCHUS, *LONGIDORUS* INTERACTION

Methods

Two experiments were set up to investigate the interaction between two nematode species, *Pratylenchus penetrans* and *Longidorus elongatus*, when feeding on a common host, *Mentha piperita* L. In the first experiment two population levels of *L. elongatus* were used. The first population contained 2, 040 nematodes per liter of soil and the second, 204 nematodes per liter of soil. The second *L. elongatus* population was diluted to a level of one-tenth the original level by adding nine parts of sterile soil to one part of inoculum containing the original level of the nematode. The initial soil population of the *P. penetrans* soil was 782 per liter of soil. The soils were mixed in a 1:1 ratio to provide a final soil population of 102 *L. elongatus* per liter and 391 *P. penetrans* per liter in treatment number one and 1, 020 *L. elongatus* per liter of soil plus 391 *P. penetrans* per liter of soil in treatment number two. The soil mixture was placed in a five inch clay pot, which holds a quart of soil, and a rooted cutting of *Mentha piperita* was transplanted into the soil. Ten replications of each treatment were prepared. The pots were watered frequently and after approximately one year in the greenhouse, plants were harvested by gently removing the soil from the soil systems while the soil was in a condition of moderate moisture. The root system of the plant and all of the soil from the can were saved for analysis of nematode content. The level of P. penetrans in the soil was determined by placing a 50 gram soil sample in a Baerman funnel. After four days a liquid sample was drawn off and counted.

The following procedure was used to determine the L. elongatus population. A 100 ml. sample of soil was sieved through a 30 mesh screen with the material flowing through this screen being subsequently strained through a 100 mesh screen. Each strainer was backwashed and the washings collected. The number of L. elongatus caught by each of the screens was counted.

The number of P. penetrans per gram of roots was determined by washing the peppermint roots thoroughly under a water tap, drying the roots with a paper towel, weighing them, cutting them into short pieces with a pair of scissors and placing them in a Waring blender. One hundred ml. of water were added to the blendor and the mixture was blended for ten seconds at low speed. The mixture was then placed on a Baerman funnel for four days and the nematodes were collected and counted. A calculation of the number of nematodes per gram of roots was made as well as a calculation of the total number of P. penetrans per pot. The latter was done by adding the number in the roots to the number in the soil.

The second experiment, which was more encompassing, was conducted using similar procedures as the first experiment. Two treatments were set up with combinations of P. penetrans and L. elongatus. The first treatment had 127 P. penetrans per liter of soil and 700 L. elongatus per liter of soil. Treatment number two had 127 P. penetrans per liter of soil and 70 L. elongatus per liter of soil. Treatments containing each nematode alone were also set up. This included a treatment of 127 P. penetrans per liter of soil, one of 700 L. elongatus per liter of soil and one of 70 L. elongatus per liter of soil. The soil was placed in five-inch clay pots. A rooted cutting of Mentha piperita was planted in each of the ten replications of the five treatments. The pots were placed in a greenhouse, watered, and fertilized to maintain healthy growth. The plants were allowed to grow for a period of approximately one year before harvest. The harvesting procedure was the same as outlined for the first experiment. When the roots were analyzed for P. penetrans a representative sample of the plant's roots was taken so that one gram of roots was processed for each plant. A one gram sample of roots was used to standardize the experimental procedure. The roots were processed as described earlier.

Results

The results of experiment one are given in Table 8. Students "t" test was used to compare the mean values of the number of

Table 8. Effect of high and low populations of *L. elongatus* on peppermint root and soil populations of *P. penetrans*. Experiment 1.

	P. penetrans per qt. soil	*P. penetrans* per gram roots	Total *P. penetrans* in roots
Longidorus 102/100 ml.			
P. penetrans 391/1.	6,494*	1,893	4,200
Longidorus 1020/100 ml,			
P. penetrans 391/1.	5,192	522	1,572
t	0.516	1.738	2.888
$t_{.10}$	1.734		
$t_{.05}$	2.101		
$t_{.01}$	2.878		
Level of significance	not significant	$t_{.10}$	$t_{.01}$

* sample mean value

Table 9. Effect of a low level of L. elongatus on the peppermint root and soil population of P. penetrans. Experiment 2.

	P. penetrans per qt. soil	P. penetrans per gram roots
P. penetrans alone, 127/ l. soil	3, 891*	1, 945
P. penetrans 127/ l. Longidorus 70/liter	2,286	1, 785
t	1. 371	0.285
$t_{.10}$	1.734	
$t_{.05}$	2.101	
$t_{.01}$	2.878	
Level of significance	not significant	not significant

* sample mean value

Table 10. Effect of a high level of L. elongatus on the peppermint root and soil population of P. penetrans. Experiment 2.

	P. penetrans per qt. soil	P. penetrans per gram roots
P. penetrans alone, 127/ l.	3, 891*	1, 945
P. penetrans 127/ l. Longidorus/700/liter	769	720
t	2.903	2.281
$t_{.10}$	1.734	
$t_{.05}$	2.101	
$t_{.01}$	2.878	
Level of significance	$t_{.01}$	$t_{.05}$

* sample mean value

Table 11. Effect of P. penetrans on a high soil population of L. elongatus feeding on peppermint. Experiment 2.

	Longidorus elongatus per 100 ml. soil
Longidorus alone, 700/liter	2, 641*
Longidorus 700/liter, P. penetrans 127/1.	1, 678
t	1.814
$t_{.10}$	1.734
$t_{.05}$	2.101
$t_{.01}$	2.878
Level of significance	$t_{.10}$

* sample mean value

Table 12. Effect of P. penetrans on a low soil population of L. elongatus feeding on peppermint. Experiment 2.

	Longidorus elongatus per 100 ml. soil
Longidorus alone, 70/liter	1, 999*
Longidorus 70/liter, P. penetrans 127/1.	914
t	3.091
$t_{.10}$	1.734
$t_{.05}$	2.101
$t_{.01}$	2.878
Level of significance	$t_{.01}$

* sample mean value

P. penetrans per quart of soil, number of P. penetrans per gram of roots and total P. penetrans present in the roots at both treatment levels. The level of significance chosen was $t_{.10}$.

The mean number of P. penetrans per quart of soil at the two treatment levels was not significantly different at the ten percent level; therefore the Null Hypothesis (H_o) was accepted. The mean number of P. penetrans per gram of roots was significantly different at the ten percent level; therefore the H_o was rejected. The mean number of the total P. penetrans in the plant roots was significantly different at the one percent level; therefore the H_o was rejected.

The results of experiment two are given in Tables 9-12. Students "t" test was again used to test the mean values of P. penetrans per quart of soil, P. penetrans per gram of roots and Longidorus elongatus per 100 ml. of soil. The level of significance selected was $t_{.10}$.

In Table 9 the mean number of P. penetrans per quart of soil at the control level and at the low level of L. elongatus was not significantly different at the ten percent level; therefore the H_o was accepted. Likewise the mean number of P. penetrans per gram of roots in the same two treatments was not significantly different at the ten percent level; therefore the H_o was accepted.

In Table 10 the mean number of P. penetrans per quart of soil at the control level and at the high level of L. elongatus was significantly different at the one percent level; therefore the H_o was rejected. The mean number of P. penetrans per gram of roots in the same two treatments was significantly different at the five percent level; therefore the H_o was rejected.

In Table 11 the mean number of L. elongatus per 100 ml. of soil at the control level of 700 L. elongatus per liter and the high level of L. elongatus mixed with P. penetrans was significantly different at the ten percent level; therefore the H_o was rejected.

In Table 12 the mean number of L. elongatus per 100 ml. of soil at the control level of 70 L. elongatus per liter and the low level of L. elongatus mixed with P. penetrans was significantly different at the one percent level; therefore the H_o was rejected.

Discussion

The results of experiment one indicate that there is an interaction between P. penetrans and L. elongatus. The fact that the number of P. penetrans within the roots of the peppermint is significantly lower when the L. elongatus level is high indicates there may be competition between the two genera for a food source. The P. penetrans level in the soil was not significantly influenced by the presence of the other genus in this experiment.

The interaction is clearly illustrated in experiment two. When the beginning level of L. elongatus was low it did not hinder the growth and development rate of the P. penetrans soil and root population. The low level of only 70 L. elongatus per liter did not provide suffi-ficient competition for the food supply to hinder the P. penetrans population. In the same replications the P. penetrans population was sufficiently high to significantly hinder the development of the L. elongatus population.

The P. penetrans population was significantly hindered in its growth and development by L. elongatus when the initial population of L. elongatus was at the level of 700 per liter. In this treatment the L. elongatus population at the higher level was hindered in its growth and development by the presence of P. penetrans. This hindrance was not as great as at the lower level of L. elongatus.

The interaction clearly exists in both directions. Generally when the level of one nematode is high initially it hindered the growth and development of the population of the other nematode genus. The population of P. penetrans in the soil and in the plant root are both influenced by the presence of L. elongatus.

Mentha piperita, commercial peppermint, is a good test host for both nematode species. The amount of food the plant provides in a clay pot is limited and the two genera are in direct competition with one another for a food supply. It is this competition which probably results in the observed population levels instead of the two populations reaching a common level regardless of the initial population.

L. elongatus is an ectoparasitic nematode which feeds upon the cortical and vascular tissue of the root from the exterior. P. penetrans is an endoparasitic nematode which feeds in the cortical and vascular region from within the plant root. P. penetrans burrows its way inside the plant root from the outside and migrates towards the vascular tissue. Norton (1958) found Pratylenchus hexinicisus Taylor & Jenkins aligned parallel to the longitudinal axis of the root and always outside the vascular elements. Townshend (1963) also described the invasion of the cortical tissues. He stated that although the endodermis was not invaded, it was the first tissue to become discolored. It is therefore evident that although P. penetrans does not invade the vascular tissue, damage is caused. P. penetrans produces a necrotic lesion in the area where it feeds. This lesion is visible to the unaided eye and if the population of P. penetrans is sufficiently high considerable damage can be done to the host plant.

The production of numerous lesion areas by a high population of P. penetrans would reduce the feeding areas available to L. elongatus and thus reduce its ability to compete for food. The result of the loss of the favorable competitive position of L. elongatus would be a slower rate of growth of the population, thus producing the results observed. The amount of food available in a lesioned area of the root would be reduced, thus forcing the nematode to migrate for further food supply. This migratory process could be detrimental for the nematode if soil conditions were not favorable for movement. A nomadic population is not likely to develop to the same level as a less migratory population because of a reduced food supply.

It is more difficult to explain the influence of a high L. elongatus population on the P. penetrans population. Competition for a food source undoubtedly plays an important role. The competition for space in the root rhizosphere also may be involved. If a large population of L. elongatus were feeding on the peppermint roots it may crowd out some of the P. penetrans which were attempting to enter the root rhizosphere with the purpose of entering the plant root. L. elongatus is a much larger nematode reaching a length of 5.5 mm. when an adult compared to the relatively small size of 0.6 mm. for P. penetrans. The size difference plus the high level in numbers may give L. elongatus a favorable competitive position for space. This competition for space as well as competition for food may explain the observed interaction between the two genera.

PRATYLENCHUS - VERTICILLIUM COMPLEX

Methods

Preparation of Verticillium Inoculum

Czapeks dox nutrient broth was prepared by adding 8.75 grams dry Czapeks dox broth and one gram of yeast extract to 250 ml. of water. After autoclaving, the broth was inoculated with *Verticillium dahliae* Kleb. No. 3 of the *Mentha piperita* strain. The flask, containing broth and inoculum, was placed on a shaker for seven days after which a level of one million propagules per ml. was reached. The shake culture was added to one pound of soil which had been sifted through a 48-mesh screen. This mixture was allowed to air dry and was resifted. The number of propagules per gram of inoculum was calculated by making a dilution series and placing samples on ethanol, streptomycin agar. Each colony produced was considered to represent on viable propagule. The prepared inoculum was used in greenhouse experiments.

Greenhouse Experiments

Two identical experiments were set up at different times with different levels of inoculum. The nematode level of the first experiment was 1,000 *P. penetrans* per quart of soil. The weight of the nematode inoculum soil added to each can along with 20 grams of *V. dahliae* inoculum was 500 grams. The final level of *V. dahliae* was 280 propagules per gram of soil. A control containing the same level of *V. dahliae* inoculum without nematodes was used. Another control with only nematodes was included.

The nematode level of the second experiment was 2,500 *P. penetrans* per quart of soil. The weight of soil per can was 250 grams plus ten grams of *V. dahliae* inoculum. The final inoculum level was 2,800 propagules per gram of soil. A control treatment with the same level of *V. dahliae* without nematodes was used.

Rooted cuttings of *M. piperita* L., were placed in each of the ten replicates of each treatment. They were grown in the greenhouse for three weeks before harvesting.

Rhizosphere Bioassay

The technique for the rhizosphere bioassay was conducted according to Lacey (1965). Plant roots were carefully removed from the cans and shaken gently, so that some soil was still clinging to the roots. This soil was considered to be the rhizosphere soil. The soil moisture level was controlled to a moderate level in all cans so that this factor would not unduly influence the amount of soil clinging to the roots.

Plant roots with adhering rhizosphere soil were placed in a 250 ml Erlenmeyer flask containing 100 ml of distilled water, which had been weighed to the nearest tenth of a gram. The flask was agitated vigorously by hand to remove the rhizosphere soil. The flask containing water, roots and rhizosphere soil was weighed to the nearest tenth of a gram. Roots then were removed, blotted dry with a paper towel and weighed. Weight of the rhizosphere soil remaining in the flask was determined by subtracting the weight of the flask plus 100 ml. water and the fresh weight of the plant roots from the total weight of flask, water, roots and soil.

After determination of the weight of the rhizosphere sample, 100 ml. of a one percent solution of carboxymethyl cellulose (CMC) was mixed with the soil suspension making a final concentration of 0.5 percent CMC. This made the suspending medium sufficiently viscous so that the larger soil particles remained in suspension while samples were taken for dilution. The mixture was vigorously shaken and samples were taken from the rhizosphere soil suspension at a constant level of one inch from the bottom of the flask.

Each rhizosphere sample was diluted to one tenth the original in a distilled water blank and one ml. of the diluted sample placed in each of five sterile petri dishes. One ml. of the nondiluted rhizosphere soil mixture was also placed in each of the five petri dishes. Ethanol-streptomycin agar, to which one gram of sodium polypectate was added, as described by Nadakavukaren and Horner (1959), was added to each plate. Each plate was agitated until the agar and rhizosphere soil were mixed. The plates were kept in the dark for 10-14 days, after which they were examined microscopically. Each colony with the typical black microclerotia of *Verticillium dahliae* was counted as one viable propagule. The results were tabulated as viable propagules of *Verticillium* per gram of rhizosphere soil. The same procedure was used for both experiments.

Results

The results of the two experiments are given in Tables 13 and 14. In experiment number one the calculated Students "t" value was 3.857. This value was considerably higher than the tabled value for 18 degrees of freedom at the one percent level, therefore the Null Hypothesis (H_o) was rejected. In experiment number two the calculated Students "t" value was 2.849. This value was higher than the table value for 18 degrees of freedom at the five percent level but was lower than the table value at the one percent level. Significance at the five percent level was accepted and the H_o was rejected. The average number of propagules per gram of rhizosphere soil was lower in experiment two but the same relationship existed between the two treatments as in experiment one. This lower number indicates a lower level of inoculum in the second experiment plus possible environmental differences due to the fact that the two experiments were run at different times.

The roots of the peppermint in the nematode plus fungus treatment were observed to have a few nematodes within them.

Table 13. The number of viable propagules of Verticillium in the rhizosphere of Mentha. Experiment 1.

Treatment	Average Number of Propagules Per Gram of Rhizosphere Soil
Pratylenchus + Verticillium	17,900
Verticillium alone	3,120
	$t = 3.857$
	$t_{.05} = 2.101$
	$t_{.01} = 2.878$

Table 14. The number of viable propagules of Verticillium in the rhizosphere of Mentha. Experiment 2.

Treatment	Average Number of Propagules Per Gram of Rhizosphere Soil
Pratylenchus + Verticillium	6,476
Verticillium alone	150
	$t = 2.849$
	$t_{.05} = 2.101$
	$t_{.01} = 2.878$

Discussion

From the results of these experiments it is evident that the presence of the root-lesion nematode, *P. penetrans*, causes a significant increase in the number of viable propagules of *Verticillium dahliae* within the rhizosphere of peppermint. This fact may be related to the increase in severity of *Verticillium* wilt when *P. penetrans* is present as has been observed by several workers (Bergeson, 1963; Faulkner and Skotland, 1965; Morsink, 1967).

According to Garrett (1956) the increase in inoculum density within the rhizosphere aids in the establishment of successful infections and progressive disease development. Garrett defines inoculum potential as the energy of growth of a fungal parasite available for infection of a host at the surface of the host organ to be infected. In this case the fungal inoculum takes the form of viable fungal propagules within the rhizosphere of the peppermint root. The potential of this inoculum may be increased either by increasing the number of infective propagules per unit area of host surface, or by increasing the nutritional status of such propagules. The results of these experiments show that the number of infective units per unit area of host surface has been increased. Although there is no evidence that the nutritional status of inoculum units has been increased, this factor also may be involved. The conclusion reached is that the presence of the nematode increases the number of viable propagule units within the rhizosphere of the peppermint plant thus increasing the inoculum potential of the fungus. This increased inoculum potential may be related to the increased incidence and severity of Verticillium wilt which has been observed by workers such as Faulkner and Skotland (1965) and Bergeson (1963).

Apart from increasing the inoculum potential of Verticillium, as previously discussed, the nematode has been postulated to have two different effects on the host making it more susceptible to Verticillium wilt. These effects are mechanical injury of the host providing a point of entry for the fungus and an unexplained physiological effect. Mountain and McKeen (1965) found that Verticillium dahliae apparently utilizes injured cortical tissue to reach the vascular elements in eggplant roots. They found that the two major sources of such root injury are damage caused by mechanically transplanting the seedlings into the field and root lesions caused by the nematode P. penetrans. The transplant injury was found to be more important early in the season and the nematode more important later in the season.

There are reports of nematodes having a physiological effect on the host plant making it more susceptible to fungal infection. Bowan and Bloom (1966) used Meloidogyne incognita (Kofoid and White, 1919) Chitwood, 1949 and Fusarium wilt to show that the influence of M. incognita was translocated. Disease symptoms developed on tomato only when both Fusarium and M. incognita both present; however, symptoms developed if the root system of tomato was split into two pots with Fusarium alone in one pot and M. incognita alone in the other pot. The nature of this translocated effect is not known.

Another author who showed that the nematode can have a physiological influence on the plant was Powell (1968). He demonstrated that Meloidogyne incognita predisposed resistant tobacco roots to invasion by species of Rhizoctonia and Pythium. The predisposition was not dependent solely on mechanical injury, but also on nematode-induced physiological changes which make host tissues more susceptible to fungal colonization. In support of this theory he showed that root-knot nematodes had to undergo a period of development within tobacco roots in order to predispose the host to fungal infection. If mechanical injury was the sole cause of predisposition the fungi would be able to colonize the host roots immediately after penetration by nematode larvae.

Faulkner (1969) demonstrated a similar response using Pratylenchus minyus [1] in combination with Verticillium dahliae on a peppermint host. He developed a technique where the two

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[1] According to Baker 1962, P. minyus is a synonym of P. neglectus (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941.

pathogens can simultaneously parasitize the plant, yet be held in isolation from one another. This was accomplished by stripping all leaves but the terminal leaves from a growing stem, passing it through a plastic tube and rooting the terminal end in another pot. Leaves were allowed to develop only at the terminal end. This double root technique has a distinct advantage over the split root method in that movement of translocatable materials is not strictly dependent upon lateral movement in stem tissues. If translocatable materials are involved, they may move freely from one root system to the other through relatively undisturbed vascular tissues.

Inoculations were carred out by adding nematode and/or *Verticillium* to the pot of each root system.

The results of his experiment showed that the nematode shortened the incubation period and increased the incidence of *Verticillium* wilt when present on either the same or a separate root system of an individual plant. *P. minyus* also affected the severity of peppermint wilt even when it parasitized a separate root system of the same plant. Wilt was more severe when plants received the fungus and nematode on separate root systems than when only *V. dahliae* was placed on one or the other root system.

The results of Falkner's experiment demonstrated that the role of *P. minyus* in the fungus-nematode complex associated with peppermint wilt is not limited to the opening of infection courts for

V. dahlia. P. minyus must induce changes in host tissues which make them either more attractive to the fungus or less tolerant of the fungus or both. That author says that it is possible that P. minyus introduces translocatable materials which have a stimulatory effect on the fungus. The nematode, while feeding, removes plant substances from host cells and also mechanically disrupts plant tissue in its immediate environment. This could readily create diffusion pressure deficits in tissues surrounding nematode lesions and increase the inflow of translocatable materials from distant portions of the plant. The author suggests that in this manner the concentration of translocatable metabolites could be reduced throughout the host. The theorized diffusion pressure deficit could explain how the translocatable materials move if they are in fact involved.

When the roots of the peppermint plants were examined for nematodes a few were found to be present but there was not suggestion of synergism such as that found by McKeen and Mountain (1960). To find such a relationship it is necessary to examine plants with fully developed symptoms. The plants used in this experiment were only two weeks old and had no visible wilt symptoms.

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