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THE ROLE OF SOIL FUNGI AND PRATYLENCHUS PENETRANS IN THE DEVELOPMENT OF STRAWBERRY BLACK ROOT ROT

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BY

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[illegible]

[illegible]

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[illegible]

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Date

To My Wife

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INTRODUCTION

In recent years the study of root diseases has shifted from a single organism approach to a study of all the organisms present in the root environment. The progress made toward a better understanding of the interactions of soil microorganisms, roots, root parasites and root pathogens has enriched plant pathology with many new ideas. When we study the below-ground pathogens, in which the several invaders may cause a complex disease, we open a new and unconventional concept in plant pathology. This is true when dealing with the black root rot disease of strawberry (Fragaria x ananassa Duch.), where the failure of the plants to grow due to brown patches or black lesions on the roots, the noticeable lack of fibrous or feeder roots, and rotting of the cortical root tissue have long been considered important factors of "strawberry degeneration".

In the decade 1930--1940 considerable attention was given to the strawberry root rot problem because of the numerous and increasing difficulties encountered by the growers in strawberry-producing areas throughout America and in Europe. The general experience of commercial strawberry growers today still shows that new beds should not be planted where strawberries have been grown previously. It is a common practice to plow up beds after one or two crops because the plants deteriorate to the extent that in the second or third year they are so weak and "spotty" that yields are

greatly reduced. Plant pathologists worked on the problem and found that many fungi would cause disease symptoms on strawberry roots. Some have reported that winter injury, viruses, fertilizer, soil type, insects, root maturity, etc. might be the causal factors. Recently, plant parasitic nematodes have been considered to play an important role in the root rot complex. Some workers have concluded that the meadow nematode is solely responsible for the discoloration and rotting of the strawberry roots.

Many incidences of black root rot have been found on strawberries throughout the state. In some cases, it has caused a complete failure of the crop. As a result, it was thought important to investigate this disease, and investigations were conducted by the writer at The University of New Hampshire during 1959, 1960, and 1961. The objective was to isolate the rhizospheric microorganisms, study their relationship to the complex problem of strawberry black root rot, and thus make a contribution to a better understanding of this disease.

LITERATURE REVIEW

Black root rot has been reported in most countries where strawberries are grown. The cause of the disease has been ascribed to various factors such as winter injury, spring frosts, cover crops and unfavorable soil, but it was generally agreed that certain parasitic fungi play a leading role in its development. As early as 1917, Fletcher (19) described a root rot or black root of strawberry. He stated that the disease was prevalent in New York, Michigan, and Massachusetts in 1902-1908. He believed that winter injury and bacterial invasion were associated with this disease. Heald (23), in 1920, mentioned the dying out of strawberries in Western Washington and ascribed the trouble to Rhizoctonia. The next year Smith and Horne (50) described a cortical root rot in California but no parasite was found. They believed that the rot was due to either water-logging or sudden drying out of the soil. In 1923 the disease was reported from Mississippi by Neal (37). A species of Fusarium was isolated from affected strawberry crowns but the fungus proved to be non-pathogenic. In Michigan, Coons (18) published a report in 1924, calling attention to the importance of black root of strawberry. He indicated that Rhizoctnia was responsible for the trouble. In the same year Sherbakoff (48) declared that black root or cortical root rot of strawberry was a disease of wide occurrence in the Southern States and that the disease was of considerable

economic importance in certain sections. He isolated a sterile fungus but was unable to identify it. About this time Berkeley and Jackson (9) began an investigation of the disease in Canada. In 1923 they reported the isolation of various soil bacteria which they thought to be pathogenic, but the next year Berkeley (4,5) found that the previously isolated bacteria failed to cause infection when inoculated into plants. A species of Fusarium was then suspected. In 1928 Thomas (54), working on the killing of strawberry roots in New York, isolated many fungi from the specimens. A Mucor-type fungus was obtained in most cases but Cephalosporium and Rhizoctonia were found also. He concluded that root killing of strawberries resulted from a number of causes, frequently in combination, and that in most cases fungi played a minor role. Bennett (3), in the same year, reported the presence of black root in Tennessee, Florida, Michigan, Washington, Wisconsin, and Ontario, Canada. In 1930 Plakidas (42) reported this disease in Louisiana. Several unidentified species of Pythium were isolated, one of which proved to be strongly pathogenic.

Investigations concerning black root rot of strawberry were carried on extensively in both North America and England during the decade from 1930-1940. In these investigations, however, no one organism was found which could be held responsible for the root rotting. Although the symptoms were quite similar, the causal organisms varied widely. The unusual feature was that no single fungus was constantly associated with it but rather several organisms were involved.

Moreover, though several of the fungi appeared to be constantly associated with black root rot when it was found, there was considerable variation in the associated groups of fungi. Sometimes, other factors could not be ignored. Therefore, black root rot was regarded as a "root-rot complex" by many pathologists. (8)

Strong and Strong (52) in Michigan showed that the Coniothyrium stage of Leptosphaeria coniothyrium (Fckl.) Sacc. and the Hainesia stage of Pezizella oenotherae (Cke. & Ell.) Sacc. (=Discohainesia oenotherae (Cke. & Ell.) Nannf.) could parasitize the roots. By pure culture inoculation, they proved the pathogenicity of these 2 fungi. In 1932, Zeller (62) described a strawberry black root rot in Oregon and demonstrated that Rhizoctonia solani Kuehn was the cause. However, when Miller (35,36) studied this disease later in the same state, he showed that under greenhouse tests the fungi which proved to be most pathogenic were Rhizoctonia, Fusarium and Ramularia. Isolates of Stemphylium, Phytophthora, Verticillium, and Pestalotia were considered to be weakly pathogenic. He stated that there was evidence of an accumulation of toxins in water-logged and poorly drained soils which would increase the susceptibility of the host. Investigations (14,40) carried out in Florida showed that Sclerotinia rolfsii Sacc., species of Diplodia and 2 species of bacteria were the causal agents for the growth failure and root rotting of strawberry plants in that state. In Louisiana (43), species of Pythium, Fusarium and Rhizoctonia were isolated from diseased strawberry roots

and proved to be more or less pathogenic. Richards and McKay (45), in Utah, reported that Rhizoctonia, Cylindrocarpon, and Obturisporium caused typical black root lesions while Hainesia was thought to be partly responsible for the disease. On the other hand, Roberts (47) reported that the blackening of strawberry roots was due primarily to winter injury. He demonstrated that if mulching was done before a temperature of $-7^{\circ}C.$ was reached, no black root injury would appear. He thought that all the fungi which were isolated from injured roots were secondary invaders. Mader (32) also believed that alternate freezing and thawing of strawberry plants was the primary cause of a physiological weakening of the plants and that the role of the fungi was only to hasten the death of the hosts. In 1935 strawberry black root was reported to be present in 15 states and was considered serious in Massachusetts, Arizona and Maryland (27).

In Canada, the investigations of this disease resulted in a series of publications. Berkeley (6) isolated species of bacteria, Fusarium, Ramularia and Rhizoctonia from diseased roots but showed in his inoculation tests that only Rhizoctonia would produce disease symptoms. Truscott (55) obtained species of Pythium, Fusarium, Alternaria, Ramularia, Rhizoctonia, Verticillium and Cylindrocladium and declared all of them to be pathogenic. He also found that under microscopic examination, Asterocystis, a plasmodiophoraceous fungus, and some phycomycetous mycorrhizal fungi which are obligate parasites of a phycomycetous

type, often were associated with strawberry roots. These fungi were also encountered by other workers later (24,25, 45,57,59). In his recent review on soil microbiology and root-disease fungi, Wilhelm (59) emphasized the importance of these phycomycetous mycorrhizal fungi or "Rhizophagus" on cortical root diseases. He stated that Rhizophagus not only inhibit root hair formation but also cause the rupturing of feeder rootlets, thus providing an entrance to the root by root-surface fungi.

During the same period "strawberry degeneration" was extensively studied in England (1,10). Berkeley and Lauder-Thomson (11), in discussing the black lesion type of strawberry root rot, contended that this disease played a major role in the general degeneration of the strawberry because of the almost constant root pruning of the host plants. They showed that species of Coniothyrium, Hainesia, Cylindrocarpon, Fusarium, and Pachybasium were pathogenic when inoculated into healthy plant roots.

According to Berkeley (7,8), the fungi connected with the strawberry root-rot complex were also associated with raspberry roots in British Columbia, and with chrysanthemums, gladioli, roses and tulips in Ontario. He stated that these root rots were the result of a complex of factors in which parasitic organisms played the leading part, and that this complex might vary with changing conditions of environment. Thus, in the case of black root rot, a temperature that favors one group of fungi may adversely affect

other groups, and therefore, temperature may be responsible in part at least for the various sequences encountered in such rots. It is true that in connection with root rot of strawberry Nolan (40) reported that the most damage by a species of Diplodia occurs when the temperature is above 27° C., while Hildebrand (8) found that black root in Ontario was favored by temperatures above 9° C.

The indirect effect of temperature and other environmental factors on the general soil flora may play an important role in favoring one group at the expense of another. Hildebrand and his associates (26,58) studied the relation of the extent and severity of black root rot to such factors as the type of cover crops preceding the planting of strawberries. They indicated that the microflora of the soil had a marked effect on the amount and severity of the root rot, although these microorganisms were not in any sense to be regarded as parasites on the strawberry roots. Plants grown in sterilized soil in which soybeans had been incorporated, remained free from disease until the third season, while those in soil following manure, corn, red clover or timothy all became diseased. In explanation of this beneficial action of soybeans, they thought that bacterial balance in the rhizosphere was important. It appeared that the biological oxidation of carbohydrate materials from decomposing soybeans in strawberry root-rot soil reduced the severity of the disease by bringing about a marked modification of the bacterial and fungal flora of the soil in such

a way that potential harmful organisms were greatly reduced and a relatively more favorable microbiological balance became established. When red clover was incorporated into the soil, a putrefactive decomposition took place with no beneficial shift from a root-rot standpoint in the biology of the soil. Katznelson and Richards (28) also demonstrated that the addition of dried blood or acetic acid to infested soil reduced the amount of strawberry root rot while oat straw increased the severity of the disease.

The time factor or sequence in which fungi become involved in the root rots of strawberry and tobacco seedlings was studied by Hildebrand and Koch (25) in Canada. It is interesting to note that in muck soil strawberry roots became infected with Thielaviopsis basicola (Berk. & Br.) Ferr. in 18 to 24 hours, whereas tobacco roots did not show the fungus until the fourth day following germination of the seed. Following this incipient infection in muck soil, Pythium sp., Rhizoctonia solani, the phycomycetous mycorrhizal fungus, orchid Rhizoctonia, nematodes and Asterocystis were observed in tobacco roots, while R. solani, phycomycetous mycorrhizal fungus, orchid Rhizoctonia and Pythium sp. were present in strawberry roots in the order given. In a strawberry root-rot soil, however, the sequence was as follows: in tobacco roots -- orchid Rhizoctonia, nematodes, R. solani, Pythium spp., phycomycetous mycorrhizal fungi, Asterocystis; in strawberry roots — R. solani, nematodes, orchid Rhizoctonia, Pythium, phycomycetous mycorrhizal fungi

and Asterocystis.

In 1948, a new disease called "Dud" was described in Washington (15). It was claimed to be responsible for over 60% of the failures of strawberries in that state. The symptoms described, however, are very close to those of black root rot. In 1950, Norton (41) reported that invasion by fungi of strawberry roots formed in the summer, did not occur until the following spring. He observed that a larger number of fungi were present in the areas of fibrous root emergence than in those areas between places of fibrous root emergence. Although many fungi were isolated, none showed pronounced pathogenicity in greenhouse tests. He concluded that the areas of root emergence might serve as avenues of entrance for microorganisms. Recently, Wilhelm and Nelson (60) found 2 new fungi which were parasitic on strawberry roots in California; later, they (39) designated one of these fungi as Idriella lunata Nelson & Wilhelm. After a pathogenicity test, Nelson (38) concluded that root deterioration of strawberry in California is caused primarily by I. lunata infection followed by invasion of the root system by secondary invaders which inhabit the rhizosphere.

By 1960, as reported in the "Index of Plant Diseases in the United States" (56), 21 fungi were listed as associated with root rot or black root rot of strawberry plants. The following fungi have been reported as capable of causing rot of cortical tissue of strawberry roots when used as inocula under certain conditions: Coniothyrium, Hainesia,

Fusarium, Rhizoctonia, Pythium, Ramularia, Cylindrocarpon, Diplodia, Sclerotinia, Cylindrocladium, Alternaria, Obtusisporium, Verticillium and Idriella. In addition, certain phycomycetous mycorrhizal fungi or Rhizophagus and plasmodiophoraceous types of fungi have been suspected of being obligate parasites.

During the last 30 years as the investigations of strawberry black root rot disease progressed, evidence has accumulated to show that plant parasitic nematodes may also play a role in this complex problem. In 1931 Steiner (51) first reported that meadow nematodes were found in strawberry roots in Florida and Massachusetts. Hildebrand (24) in 1934 noted that nematodes were frequently and consistently associated with diseased strawberry roots and suggested that nematodes might be the cause of strawberry root rot. Later Hildebrand and West (26,58) identified the nematodes associated with strawberry root rot as Pratylenchus pratensis (De Man) Fillip. Hastings (22) reported that in British Columbia a root decline of narcissus and strawberry roots was caused by meadow nematodes. Bosher (12) later identified those meadow nematodes from British Columbia as P. pratensis and those from Vancouver Island as P. penetrans Sher & Allen. In England, Berkeley and Lauder-Thomson (11) also found numerous nematodes in the fine roots of strawberry during the early spring. They stated: "the possibility of nematodes being a factor in the root rot complex should not be overlooked." However, they did not include nematodes in

their inoculation test.

There have been many reports of nematode damage to strawberry roots in the United States. Braun (13) in 1958 conducted a survey of plant parasitic nematodes associated with strawberry roots and showed that Pratylenchus spp. were the most prevalent forms in this country. Klinkenberg (30) also found meadow nematodes associated with black root rot of strawberry in The Netherlands. Later in an inoculation experiment with meadow nematodes (P. penetrans) and strawberry seedlings, she presented further evidence to support the belief that nematodes must be present before soil fungi in the rhizosphere are able to attack strawberry roots. Thus she concluded that P. penetrans is the primary cause of black root of strawberry plants. Goheen and Smith (20) reported that P. penetrans could enter strawberry roots directly and feed and reproduce therein. The roots of strawberry plants from nematode-infested soil were badly rotted and showed typical black root rot symptoms, whereas those from nematode-free soil were almost free of rot. Riggs, et al (46) studied P. coffeae (Zimm.) Sher & Allen in relation to decline of strawberry plants in Arkansas, and declared that this meadow nematode was associated with black root rot.

According to Allen and Raski (2), field tests for the control of black root rot of strawberries by soil fumigation resulted in improved growth which was also correlated with the control of P. penetrans. But later, when P. penetrans was tested on strawberry plants grown in black-root-rot soil

in the greenhouse, Raski (44) concluded that in California this nematode was probably not the most important factor limiting the growth of strawberries affected with black root. In Connecticut, Miller (33) reported that the damage to strawberry roots by fungi or nematodes might be independent of the presence of the other. Therefore, soil treatments with both fungicides and nematocides might give better control of black root rot of strawberries than either of them used alone.

The problem became more complex when Skilès (49) in Minnesota reported that a strawberry virus played a role in the root rot complex. He showed that when strawberry plants inoculated with the Xanthosis virus, were inoculated with Rhizoctonia sp., their roots were rotted considerably more than those on virus-free plants. Moreover, Taper, et al (53) in Canada studied the nutrition of the strawberry in relation to black root disease and showed that boron toxicity was accompanied by certain symptoms characteristic of black root rot.

EXPERIMENTAL PROCEDURE AND RESULTS

Section I. Preliminary Survey of the Disease

Materials and methods.--Eight popular commercial strawberry varieties (cultivars) were sampled from 12 locations in the state during the growing seasons of 1958 and 1959. Plants were collected from several sites in a given field. Particular attention was given to those plants which showed lack of vigor, wilting, or the prospect of dying prematurely. Plants were carefully dug to avoid breaking off the smaller roots. They were labeled and stored in 1-quart polyethylene bags in a refrigerator at 4° C. until examination.

Plant roots were thoroughly washed in a stream of running tap water. At least 10 plants of each cultivar from each of the plantations were examined for the presence of black root rot. This was done for the purpose of estimating the incidence of the disease for the state as a whole.

Fungus isolations were made from roots exhibiting lesions or rot. Single roots as well as pieces of root containing lesions were plated. Various methods of disinfesting root surfaces were tried, since the roots were so small and tender sterilization with chemicals such as mercuric chloride usually resulted in killing every microorganism in and on the roots. Rinsing root pieces in running tap water for 1 hour, then transferring directly into plates usually re-

sulted in a high frequency of bacterial growth. Consequently, the following method was found satisfactory and used as a standard method in later experiments for the isolation of fungi from strawberry roots. This consisted in cutting off root sections 3-8 mm long, with each section containing a rot lesion and a portion of healthy tissue. Root pieces were placed in a plastic mesh basket, rinsed with distilled water for 10 minutes and then submerged in a 5% Clorox (sodium hypochlorite) solution for 3 minutes. They were rinsed again with sterilized distilled water, then 5 sections were transferred to an agar plate by means of flame sterilized forceps. Four types of agar medium were tried: water agar, V-8 juice agar, potato dextrose agar and potato dextrose plus yeast extract agar (35 g PDA + 2 g yeast extract / 1 L water). It was found that most of the fungi isolated grew better on potato dextrose-yeast extract agar than they did on other media. Therefore, all fungal isolations were made on this medium.

After plating, various incubating temperatures were tried in order to obtain a temperature favorable for growth of most of the fungi. A temperature of 18° C. was found to be feasible. Fungus colonies usually appeared in 5 to 14 days depending on the growth rate of different types. The hyphal tips of each isolate were transferred to petri dishes containing the same type of agar medium. Later, all isolates were grouped according to type, and each type was assigned a number before identification. The pure isolates were kept

in tubes containing potato dextrose-yeast extract agar slants for later use.

All sampled roots were checked for the presence of plant parasitic nematodes. Techniques described by Young (61), Caveness and Jensen (16), Miller (34), and Baermann (21) were tried. It was found that if plant roots were reduced to small pieces, the nematodes could work free easily, and thus the Baermann funnel technique was found just as useful to extract nematodes from roots as from soil samples. Roots were also occasionally stained with lactophenol-acid fuchsin to determine the presence of nematodes.

The extracted nematodes were picked up with a No. 1 Pulp Canal Reamer mounted in a glass tube, and were transferred to slides and placed under a microscope for examination. Specimens were also transferred in formalin fixative (5 ml stock formalin + 32.5 ml water) to a slide, and the cover-glass sealed carefully with Gold Seal. These slides were then sent to Mr. A.L. Taylor, Nematologist, U.S. Department of Agriculture, Beltsville, Md. for identification.

In 1959, cultivated and wild plants grown near or within strawberry fields were checked for the incidence of meadow nematodes. The processing and extraction were done in the same way as described before.

Results.--A survey of 12 strawberry fields widely distributed in New Hampshire with equally wide diversity of soil types and strawberry varieties revealed that black root rot was present in all the plantations visited. The inci-

dence and severity of black root rot at different locations as well as the isolation of fungi and nematodes from the sampled roots are shown in Table 1. The strawberry field in Londonderry was visited in the summer of 1959. Almost every plant showed stunted growth with dwarfed leaves and petioles. These plants were set in the field early in the spring, but most of them produced only 1 or 2 runners. Many plants had already died, leaving empty spaces in the rows. Root rots were commonly found, but undoubtedly they were not the main reason for inhibiting plant growth because in many plants no indications of root rot could be found. Later it was found that these plant roots harbored a very high population of meadow nematodes, mainly P. penetrans.

Strawberry plants sampled from University experimental nurseries in Durham showed that meadow nematodes could be recovered from roots and associated soil at any time of the year. Black root rot was also commonly found but the degree of rotting was always low. In another survey at Northwood, black root rot was undoubtedly the main, if not the sole cause of the failure. However, nematode recovery was relatively low from those samples.

Of all the plant parasitic nematodes recovered from strawberry samples, P. penetrans was the predominant nematode species. Also Aphelenchoides Fisher, and Ditylenchus Filipjev were each encountered in 1 sample.

It was realized after plating the lesioned root sections that a large number of apparently different fungi

Table 1. - Results of survey[a] showing the distribution of black root rot, plant parasitic nematodes and fungi associated with strawberry roots in New Hampshire.

Location	No. strawberry var. sampled [b]	Relative abundance of nematodes [c]	Fungus isolation No. sections plated	Fungus isolation No. fungi isolated	Incidence of black root rot [c]
Hillsboro	1	+++	---	---	+
Madbury	5	+	120	98	+
Durham	2	++	---	---	+++
Plymouth	6	+	300	321	++
Boscawen	2	+	---	---	+
Londonderry	3	++++	305	256	++
Piermont	5	++	---	---	+
Durham (UNH)	1	++	190	145	++
Durham (Rich)	1	+	250	278	+++
Northwood	4	+	50	58	++++
Nottingham	2	++	40	41	++
Durham (Tecce)	6	+	150	97	++

[a] During the growing seasons of 1958 and 1959.

[b] Strawberry cultivars or varieties investigated were: Premier, Catskill, Sparkle, Robinson, Great Bay, Blaze, Strafford and Phelps.

[c] +=few; ++=moderate; +++=high; ++++=very high.

could be obtained. The same types of fungi could be isolated from roots of different strawberry varieties or cultivars. Moreover, the same types of fungi were also found from roots of samples obtained from different locations and at different seasons of the year. It indicated that some parasitic fungi were consistently associated with rot lesions. In addition, there appeared to be no indication of differences in strawberry varietal susceptibility to the invasion of fungi, nematodes, or the presence of the black root-rot disease.

The survey of many cultivated and wild species of plants in 1959 was made to verify early reports that meadow nematodes have a wide host range. As can be seen in Table 2, Pratylenchus spp. can attack and enter the roots of many plant species. Therefore, many kinds of plants could act as reservoirs for meadow nematodes which could become potential inoculum to nearby or succeeding strawberry crops.

Table 2. - Meadow nematodes (Pratylenchus spp.) associated with some wild and cultivated plants investigated near or in strawberry fields in New Hampshire.

Crop	No. locations investigated	Relative abundance of meadow nematodes [a]
Alfalfa	2	++
Apple	1	++
Asparagus	1	--
Broccoli	3	+
Cabbage	3	++
Cauliflower	3	+
Chickweed	2	+++
Chinese Cabbage	2	+
Field corn	2	+++
Oats	2	+++
Orchard grass	2	+++
Pansy	1	+
Pea (garden)	1	++
Red clover	3	+++
Sweet corn	3	+
Tomato	2	++
Vetch	2	++
White clover	3	++++
White pine	1	--
Wild mustard	3	+++

[a] --=none; +=few; ++=moderate; +++=high; ++++=very high.

Section II. Experiments with Fungi

Materials and methods.--Two strawberry fields were selected for sampling the roots and isolating fungi from them. Strawberry plants were collected 3 times in 1959 and 4 times in 1960 from a plantation in Londonderry known to be heavily infested with *P. penetrans*. In 1960 samples were collected from a University experimental nursery in Durham approximately every 2 weeks from April to December. At both locations 20 plants each of 2 cultivars, Premier and Sparkle, were taken randomly in the field. They were stored in polyethylene bags in a refrigerator. The composite root samples of each cultivar were examined and diseased sections were removed. They were surface-sterilized and plated on potato dextrose-yeast extract agar medium. Isolates which appeared to be the same were grouped as described before.

In view of the number and diversity of fungi isolated, a quick test for pathogenicity of each isolate would be of great advantage. Kilpatrick (29) applied a test tube technique to determine the pathogenic effect of various fungi on clover seedlings. This technique was tried with strawberry seedlings without success. Strawberry seeds need a relatively long period to germinate and their roots develop much more slowly than clover roots. A technique was then developed for growing strawberry seedlings on a nutrient agar medium in test tubes. The tubes were placed in test tube racks, and the seedlings were grown under a fluorescent

light containing two 40-watt daylight-type lamps. A minimum photoperiod of 16 hours was necessary, but plants grew just as well under constant illumination. Seedlings could grow in the test tube for as long as 6 months without refilling the tubes with nutrient medium.

Strawberry seeds were stored in glass vials at 4°C. They were scarified for 15 minutes in concentrated sulfuric acid, rinsed in running tap water for 30 minutes, then surface-sterilized with 10% Clorox (sodium hypochlorite) solution for 5 minutes and rinsed once again with sterilized distilled water. They were then transferred into sterile petri dishes on moist filter papers and allowed to germinate at room temperature. Ten to 14 days later, the tiny seedlings were aseptically transferred to test tubes (150 x 18 mm) half filled with sterile nutrient medium. The medium used was a modified Hoagland's and Knop's solution, prepared as follows and adjusted to a pH of 6.0:

Macroelements

$Ca(NO_3)_2 \cdot 4H_2O$	0.95 g
KNO_3	0.61 g
$MgSO_4 \cdot 7H_2O$	0.49 g
$NH_4H_2PO_4$	0.12 g

Microelements (Stock solution - 1000 ml in vol.)

$MnSO_4 \cdot 4H_2O$	3.00 g
$ZnSO_4 \cdot 7H_2O$	0.50 g
H_3BO_3	0.50 g
$CuSO_4 \cdot 5H_2O$	0.025 g

$Na_2MoO_4 \cdot 2H_2O$	0.025 g
H_2SO_4 (sp. gr. 1.83)	0.50 ml

Ferric Citrate (Stock solution - 1000 ml in vol.)

$FeC_6O_5H_7 \cdot 5H_2O$	10.00 g
Agar	5.00 g
Distilled water	to 1000.00 ml

One ml of the microelement stock solution and 2 ml of the ferric citrate stock solution were used for each liter of medium.

When strawberry seedlings had become established in the tubes, usually with 4 true leaves fully expanded and with root system well developed, a pure culture of a previously isolated fungus was transferred to the agar medium. Four test tubes with 4 seedlings of relatively uniform size constituted 1 treatment. Seedlings which received sterile agar discs served as controls. All tubes were arranged randomly in racks.

The progress and the development of the disease symptoms were observed daily by means of a dissecting microscope (X 15 and X 45). Each plant root was scored on the basis of root discoloration at 10, 14, and 21 days after inoculation. The rating system was as follows: 0, no evident symptoms either macroscopically or microscopically; 1, less than 25% of the root system slightly discolored; 2, 26-50% of the root system slightly to moderately discolored; 3, 51-75% of the root system discolored, ranging from slight to severe; 4, over 75% of the root system moderately to severely rotted. The cumulative ratings were summed for

each treatment and the total value was divided by the number of plants in each treatment, i.e., 4. The average cumulative rating per plant at the scoring date was used for comparing pathogenicity of different fungal isolates. This reflected the relative rapidity of development of the pathogenic effect of a specific fungus as well as the final rating of the rot. Those fungi which caused an average final cumulative rating of 2.0 or above were selected for further tests.

To ascertain if all the selected test-tube pathogenic fungi (TPF) were also pathogenic to strawberry roots in a semi-natural condition, 2 experiments were conducted in the greenhouse in 1960 and 1961. In 1 test, Premier strawberries (virus free) were collected from University plots and transplanted to 4-inch pots in the greenhouse. Fertilizer (16-32-16) was applied to these plants every 2 weeks and illumination was given to provide at least a 17-hour photoperiod. All plants produced many runners which were "caught" in sterilized 3-inch pots filled with steam-sterilized vermiculite (Fig. 1). Three weeks after rooting, the plants with uniform size were selected and were severed from mother plants. Each pot with 1 newly rooted runner plant was set in a 4" x 4" x 2.5" plastic container and watered daily with distilled water. A pure culture of the TPF to be tested was increased on potato dextrose-yeast extract agar in 10 cm petri plates. Three plates of each TPF pure culture were added to 200 ml of distilled water in a

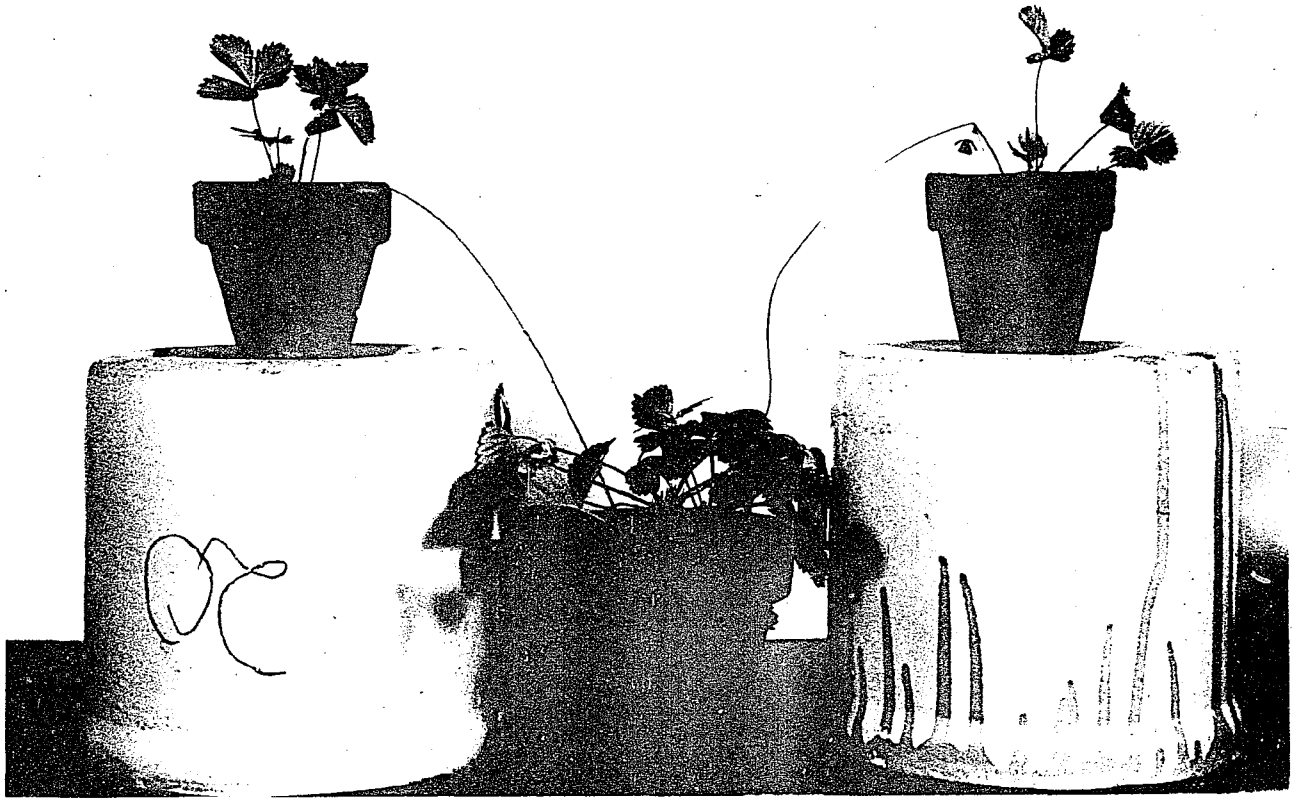

Figure 1. - Strawberry mother plant and runner plants - showing a method of obtaining fungus-free and nematode-free plants in the greenhouse.

Waring Blendor and macerated for 30 seconds. Aliquots of the resulting mixture of each TPF were poured into 4 plastic containers. An uninoculated agar suspension was used for the check plants. They were then arranged randomly on a greenhouse bench and watered daily with distilled water. Six weeks after inoculation, plants were removed from the pots and the roots were washed in running tap water before final notes were recorded.

In another experiment the TPF inocula were prepared in the same way but aliquots of each fungus were poured into five 3-inch pots containing steam sterilized sandy-loam soil. The fungi were allowed to become established in the pots for 2 days before strawberry plants were transplanted. Uniform 10-week old strawberry seedlings of the cultivar Sparkle (open pollinated) were used in this test. They were removed from 2-inch pots and thoroughly washed in running tap water. The root system of each plant was cut back to about 1 inch from the crown, rinsed in distilled water and transplanted in the pot containing fungus infested soil. All plants were arranged randomly on a bench in the greenhouse. Resulting data were taken 4 weeks later.

Perhaps the most difficult phase in the study of pathogenicity of fungi to strawberry roots was that concerned with arriving at a proper way to evaluate the degree of rot. Newly initiated roots usually appeared white or light tan in color and thus it was easy to detect the presence of rot lesions. If, however, a plant consisted of

many corky roots the fine fibrous roots constituted the only region suitable for checking rots. Therefore, the rot ratings in greenhouse tests were obtained by randomly selecting 5 roots of each plant and scoring each root according to the degree of discoloration. 0 represents no discoloration, while 4 represents complete blackening of the root. The average rating of the 5 roots represents the degree of rot for that plant.

Results.-- A total of 3865 fungal isolates was obtained from 960 sampled strawberry plants at 2 locations in 1959 and 1960 (Table 3). It was found that the total number of fungi isolated from Premier roots differed little from Sparkle if they were sampled in the same strawberry field and at the same time. The frequency of recovery of several fungi differed very little between these 2 cultivars. All isolates obtained from the Londonderry field belonged to the various fungal groups isolated from the Durham field. These isolates were grouped into 107 types representing at least 96 genera.

These 107 fungal-groups were tested individually for pathogenicity on strawberry seedlings (Sparkle--open pollinated) in test tubes. Table 4 shows the rapidity of pathogenic effect of each specific fungus on strawberry roots and the final rot rating of the roots. A total of 27 fungi was selected based on the final ratings at the 21st day (see Appendix). Note that most of these test-tube path-

Table 3. -.Number of strawberry root sections plated and fungus isolates obtained from samples collected at 2 locations during 1959 and 1960.

Source of samples	No. of samples	No. of plants examined	No. of sections plated	No. of fungi isolated
Londonderry	7	280	505	505
Durham	17	680	3400	3365
Total	24	960	3905	3865

Table 4. - Relative pathogenicity test of fungi isolated from strawberry roots sampled from 2 locations in New Hampshire, during 1959 and 1960, -- based on test-tube technique. [a]

Isolated fungal type number	Degree of root rot [b] 10 days	14 days	21 days
1	0	0	0.25 [c]
2	0	0	0.50
3	0.25	1.75	3.50
4	0	0.25	0.75
5	0	0	0
6	1.25	2.00	3.25
7	0	0	0.50
8	1.00	1.50	2.25
9	1.00	1.25	2.25
10	0	0	0
11	1.00	1.50	2.25
12	1.25	2.25	3.50
13	0.50	1.50	2.25
14	0.50	0.75	1.00
15	0	0	0
16	0.25	0.25	0.25
17	0	0.50	0.50
18	0.50	1.50	2.00
19	0.25	1.00	1.00
20	0.75	1.25	2.00
21	0	0.75	0.75
22	0	0	0
23	0	0	0
24	0	0.50	1.00
25	0.50	1.50	2.25
26	0	0.75	0.75
27	0.25	0.75	0.75
28	0	0.50	0.75
29	0	0.50	0.50
30	0.25	10..	2.25
31	0	0.50	1.00
32	0	0	0.25
33	0	0.25	0.50
34	0.50	1.25	2.50
35	0	0	0
36	0	0.50	0.50
37	0	0	0

Table 4. -- Continued

38	0	0	0
39	0	0	0
40	0	0.75	1.00
41	2.00	2.75	3.75
42	1.50	2.50	2.25
43	0	0	0
44	0	0	0.25
45	0	0.50	0.75
46	0	0	0
47	2.25	3.00	4.00
48	1.00	2.25	3.50
49	0	0	0
50	0	0.50	0.50
51	0	0.50	0.75
52	0	0	0
53	0	0	0.25
54	0	0	0
55	1.75	2.50	4.00
56	1.00	2.00	2.75
57	0	0	0.25
58	0	0.25	0.75
59	0	0	0
60	0.25	0.25	0.50
61	0	0	0
62	0	0.50	2.00
63	0	0	0.50
64	0	0	0
65	0	0	0
66	0	0.25	0.50
67	0	0	0
68	0	0	0
69	0	0	0
70	1.00	1.50	2.25
71	0	0	0
72	0	0	0
73	0	0.25	0.50
74	0	0	0
75	0	0	0.25
76	0	0	0
77	0.25	0.75	2.00
78	0	0	0
79	0	1.00	2.25
80	0	0.50	0.50
81	0	0.25	0.50
82	1.00	2.75	4.00
83	0	0.50	0.50
84	1.00	2.50	3.00
85	2.25	3.75	3.75
86	0.50	1.50	2.00
87	1.00	1.50	2.00
88	0	0	0

Table 4. -- Continued

89	0	0	0
90	0	0.50	0.75
91	0	0	0
92	0.50	0.50	0.50
93	0	0	0.25
94	0	0	0
95	0	0.25	0.25
96	0	0.50	1.00
97	0	0	0
98	0	0	0
99	0	0	0
100	0	0.25	0.25
101	0.25	0.25	0.25
102	0	0	0
103	0	0.50	0.50
104	0	0.25	0.25
105	0	0	0
106	0	0.50	0.75
107	0	0	0.25

---

a/ Fungi causing a final root rot rating of 2.00 or above are considered to be pathogenic.

b/ 0= no discoloration; 1= 1-25%; 2= 26-50%; 3= 51-75%; 4= 76-100%.

c/ Each figure represents an average of 4 seedlings.

ogenic fungi (TPF) caused an average rot rating of 1.00 or more by the 10th day. It was also noted that several very weakly parasitic fungi could cause a little discoloration on roots by the 10th day but the rot did not progress further.

TPF were used to compare with the total number of isolated fungi from 2 locations in 1959 and 1960. The results are given in Tables 5 and 6. It was noted that 75.2% and 70.5% of the total fungal isolates belonged to the TPF group. There was among the TPF a great diversity of relative prevalence ranging from less than 0.1% to 20.2%. Since only 7 samples were collected in the Londonderry field and only 505 isolates were obtained, it was logical to conclude that some of the fungi which occurred least frequently have very little chance to be encountered there. However, the most prevalent fungi isolated from Londonderry were comparable to those isolated from Durham. Tables 7 and 8 demonstrate the relative prevalence of TPF isolated from Durham and Londonderry respectively. TPF No. 9 was the most prevalent type isolated at both locations. Types 8, 18, 82, 85, 3, and 6 were also consistently associated with lesioned roots. However, those TPF which were most frequently encountered did not always coincide with their relative pathogenicity. As shown in Table 4 the highest rot rating was caused by TPF 47, 55, and 82 but their occurrence in the percentage of total isolates, as shown in Table 7, were 0.2, 0.5 and 9.3, respectively. TPF 8 and 9 comprised 11.4% and

Table 5. - Comparative populations of pathogenic and nonpathogenic fungi associated with discolored strawberry roots from Durham field plots, April to December, 1960.

Sample date	Sections plated	Total No. of isolates	No. of pathogenic fungi [a]	% of pathogenic fungi
4/14	200	78	45	57.7
4/20	200	123	89	72.4
5/3	200	192	144	75.0
5/18	200	197	144	73.1
6/4	200	213	166	78.0
6/16	200	211	154	73.0
7/1	200	203	154	75.9
7/17	200	243	203	83.5
7/29	200	242	181	74.8
8/12	200	238	159	66.9
8/25	200	192	110	57.3
9/9	200	200	142	71.0
9/23	200	168	111	66.1
10/7	200	203	167	82.3
10/21	200	220	166	75.5
11/11	200	264	246	93.2
12/2	200	178	151	84.9
Total	3400	3365	2532	75.2

[a] Based on the results obtained from fungus pathogenicity tests in test tubes.

Table 6. - Comparative populations of pathogenic and non-pathogenic fungi associated with discolored strawberry roots from a commercial field plot at Londonderry, New Hampshire.

Sample date	Sections plated	Total No. of isolates	No. of pathogenic fungi [a]	% of pathogenic fungi
1959				
8/26	150	124	78	62.9
10/4	155	132	87	65.9
11/3	40	43	37	86.0
1960				
5/10	40	36	28	77.8
6/3	40	44	34	77.3
7/22	40	48	27	56.3
9/15	40	78	65	83.3
Total	505	505	365	70.5

[a] Based on the results obtained from fungus pathogenicity tests in test tubes.

Table 7. - Relative prevalence of pathogenic fungi[a] isolated from discolored strawberry roots from Durham field plots, April to December, 1960.

Fungus number	Times isolated[b]	% of total isolates
9	677	20.1
8	382	11.4
82	312	9.3
85	240	7.1
18	218	6.5
3	172	5.1
25	80	2.4
6	78	2.3
34	50	1.5
11	33	1.0
41	29	0.9
87	28	0.8
30	26	0.8
12	24	0.7
20	21	0.6
79	19	0.6
70	18	0.5
13	18	0.5
48	17	0.5
55	16	0.5
42	15	0.5
77	11	0.3
86	8	0.2
47	6	0.2
62	3	0.1
56	1	--- [c]
Total	2532	75.2

No. of non-pathogenic types	Total No. isolated	% of total isolates
80	833	24.8

[a] Based on the results obtained from fungus pathogenicity tests in test tubes.

[b] Represent the total number isolated from 3400 root sections of 17 surveys.

[c] Less than 0.1%

Table 8. - Relative prevalence of pathogenic fungi[a] from discolored strawberry roots from a commercial plot at Londonderry, New Hampshire, 1959-1960.

Fungus number	Frequency of isolation[b]	% of total isolates
9	87	17.2
18	67	13.3
82	54	10.7
8	42	8.3
85	30	5.9
6	27	5.4
3	27	5.4
11	12	2.4
20	2	0.4
12	1	0.2
13	1	0.2
34	1	0.2
48	1	0.2
70	1	0.2
79	1	0.2
84	1	0.2
87	1	0.2
Total	356	70.5

No. of non-pathogenic types	Total number isolated	% of total isolates
46	149	29.5

[a] Based on the results obtained from fungus pathogenicity tests in test tubes.

[b] Represent total number isolated from 505 root sections of 7 surveys.

20.1% of the total isolates while in tubes they both affected the roots, causing an average rot rating of only 2.25.

The results from inoculating newly rooted strawberry runner plants with pure cultures of 27 TPF and the percentage of recovery from roots are given in Table 9. They indicate that most of these isolates were able to produce root rot to a certain extent, although the rot ratings were lower than those from test tube results. Soil contamination and subsequent root contamination during a long period in the greenhouse was inevitable. Trichoderma spp., Fusarium spp., Penicillium spp. and Alternaria spp. comprised over 85% of the total contaminating fungi. This may explain the failure to recover several TPF in the test and the presence of root discolorations in such treatments as well as in the uninoculated check plants.

Isolate No. 9 produced the highest rot rating in this experiment, followed by 34 and 41. No. 8 was recovered in the highest percentage, followed by 82, 6, 18, and 12. Strawberry plants showed stunting only when their roots were very severely rotted. Hence, there were variations of plant vigor among and within treatments. The uninoculated control plants, nevertheless, were the most uniform and vigorous group in spite of some root discoloration.

The results of the second greenhouse experiment for testing TPF pathogenicity are shown in Table 10. Since the pruned roots became almost completely rotted in every

Table 9. - Greenhouse test of fungus pathogenicity.

Experiment No. 1.

Fungus Number	Root rot rating [a]	Sections plated	Total No. fungus isolates	No. times inoculum recovered	% recovery of inoculum
3	0.19	20	11	3	27.3
6	1.35	20	6	4	66.7
8	1.25	20	21	19	90.5
9	2.60	20	12	4	33.3
11	1.45	20	4	1	25.0
12	1.65	20	6	3	50.0
13	0.45	20	3	0	----
18	1.50	20	14	7	50.0
20	1.40	20	16	1	6.3
25	1.80	20	37	14	37.8
30	0.80	20	14	0	----
34	2.45	20	31	11	35.5
41	2.25	20	15	4	26.7
42	1.80	20	21	3	14.3
47	1.80	20	14	1	7.1
48	1.35	20	11	3	27.3
55	2.00	20	29	8	27.6
56	0.25	20	7	0	----
62	0.95	20	20	0	----
70	0.75	20	13	3	23.1
77	0.70	20	9	2	22.2
79	0.30	20	9	2	22.2
82	1.50	20	10	7	70.0
84	0.35	20	8	0	----
85	1.30	20	13	5	38.5
86	1.35	20	14	4	28.6
87	0.50	20	12	0	----
Check	0.23	80	15	--	----

[a] Each figure represents an average of 4 plants. 0=healthy; 1=1-25% root rot; 2=26-50%; 3=51-75%; 4=76-100%.

Table 10. - Greenhouse test of fungus pathogenicity.

Experiment No. 2.

Fungus number	Root rot rating [a]	Sections plated	Total fungus isolates	No. times inoculum recovered	% recovery of inoculum
3	1.32	20	23	20	87.0
6	0.68	20	22	0	----
8	1.00	20	22	20	91.0
9	1.80	20	23	14	60.9
11	1.24	20	25	18	72.0
12	1.08	20	21	4	19.1
13	0.60	20	22	2	9.1
18	0.52	20	34	9	26.5
20	1.12	20	27	7	25.9
25	0.60	20	30	17	54.8
30	0.36	20	31	13	41.9
34	1.40	20	32	12	37.5
41	1.40	20	18	2	11.1
42	0.94	20	22	2	9.1
47	0.32	20	23	3	13.0
48	0.60	20	22	2	9.1
55	1.68	20	21	12	57.1
56	0.60	20	19	5	26.3
62	0.40	20	24	13	54.2
70	0.68	20	23	17	73.9
77	0.92	20	22	16	72.7
79	0.72	20	23	10	43.5
82	1.12	20	20	16	80.0
84	0.36	20	17	2	11.8
85	1.12	20	23	20	87.0
86	0.56	20	23	12	52.2
87	0.48	20	33	11	33.3
Check	0.32	40	14	--	----

[a] Each figure represents an average of 5 plants. 0=healthy; 1=0-25% root rot; 2= 26-50%; 3= 51-75%; 4= 76-100%.

treatment, only the newly developed roots were checked and scored at the end of the test. Attempts were made to plate a few sections of the old, rotted roots from each treatment. Over 95% of the fungi recovered were the same as the inoculum. This indicated that the old weakened roots were more susceptible to TPF invasion than newly initiated roots. In addition, these old roots could harbor fungal pathogens and be a constant threat to the neighboring healthy roots. This might explain the higher percentage of TPF recovery in this experiment than in the previous one.

Completely blackened new roots have not been observed in this experiment but patches of discoloration were commonly found in every treatment. The symptoms were similar to those observed on new roots during early spring or late fall in the field.

No. 9 fungus again produced the highest rot rating, followed by 55, 41, 34, 3, and 11. No. 8 was again recovered in the highest frequency, although in both experiments this fungus did not produce a high score for rot. The only fungus in this test which was not recovered was No. 6. The reason for this is not known because this fungus comprised 66.7% of the isolates in the first experiment.

The relative prevalence of fungi in both greenhouse experiments coincided closely with field isolation data as shown in Tables 7 and 8. Except for No. 9, all fungi which occurred frequently in field samples were also reisolated from roots in higher percentages in greenhouse tests.

Section III. Experiments with *Pratylenchus penetrans*

Materials and methods.-- According to the preliminary survey, *P. penetrans* was the most prevalent plant-parasitic nematode in association with strawberry roots in New Hampshire. It was considered desirable that additional investigations on this nematode in relation to strawberry black root rot should be made.

Two strawberry fields selected for studying root rotting fungi were also used for nematode investigations. A total of 8 samples were collected from the Londonderry field during 1959 and 1960, and 17 samples were collected from the Durham field in 1960. Except for 1 sample that was collected from Londonderry in July, 1959, the other sampling dates were the same as those for fungus studies. Two cultivars, Premier and Sparkle, were selected for studying the seasonal variation of nematode population in both fields. Twenty plants of each cultivar were dug on each collection date.

Roots were washed to remove soil and debris and were surface-dried by blotting with paper towels. The composite roots of each cultivar were chopped with a pair of scissors into small pieces (1/3 - 1/2 cm long) and mixed together thoroughly. Four 4-gram representative samples were each placed in a modified Baermann funnel (125 mm) on a piece of 1-layer Scott facial tissue paper which was supported by a copper wire screen. The funnel was filled with

distilled water until the level was just above that of the roots, and was covered with half of a petri dish (150 x 15 mm). After 24 hours at room temperature, a 50 ml sample from each funnel was drained into a beaker. The funnels were refilled with distilled water and the same process was repeated 24 hours later. Three 10-ml aliquots from each beaker were examined and counted using a counting plate. An average of these counts was then recorded and the number of nematodes present in each gram of roots at a given date could be calculated.

The nematodes were extracted from a 100-gram soil sample adjacent to the roots by means of sieve and Baermann funnels according to the method described by Christie and Perry (17). Counts were made in the same manner as described for the root samples.

Parasitism and pathogenicity of P. penetrans on strawberry roots were studied in the greenhouse as well as in the laboratory. Two tests were conducted in the greenhouse in 1960 and 1961. In the first experiment, nematode inocula were obtained from strawberry roots from a heavily infested field in Londonderry. Roots were separated from soil, then washed and cut into small pieces. They were mixed with steam-sterilized soil and placed in 4-inch pots. Four garden pea (Pisum sativum L.) seedlings were transplanted into each pot and allowed to grow for 2 months. The pea roots were harvested, thoroughly rinsed with running tap water, mixed and weighed. A few samples were taken from

the composite roots to determine the number of P. penetrans present in each gram of roots. They were added to the pots where newly rooted Premier strawberry runner plants were growing. (The method of obtaining runner plants was described in section II. --"Experiments with Fungi")

Three 10-plant treatments were set ut with the addition of 2000, 1000, and no nematodes to each plant in each treatment. One 4-inch pot containing 1 plant grown in steam-sterilized sandy loam soil was set in another 4-inch pot which contained approximately 1 inch of steam-sterilized sand. All treated plants were arranged randomly on a greenhouse bench.

In the second greenhouse experiment, the nematode inoculum used was a pure culture of P. penetrans reared on undifferentiated clover root callus, growing in vitro, on a modified culture medium described by Krusberg (31). This species of meadow nematode was originally obtained from Ladino white clover roots. The nematodes were hand picked and disinfested by immersion in a solution of streptomycin sulfate (0.1%) and malachite green (30 ppm) for 4 hours. They were rinsed in sterilized distilled water and then transferred to tubes (150 x 18 mm) containing clover callus tissue on an agar medium. To prevent dehydration of the medium and plant tissues each tube was covered with a sterilized aluminum foil cover and sealed with Scotch cellophane tape. Tubes were kept in darkness at room temperature. Nematodes were allowed to multiply in the tubes and were transferred

to new callus tissues whenever necessary.

The modified medium for clover root callus culture was prepared as follows:

Solution A.

$Ca(NO_3)_2 \cdot 4H_2O$	0.50 g
KNO_3	0.12 g
$MgSO_4 \cdot 7H_2O$	0.12 g
KH_2PO_4	0.12 g

Solution B. (Stock solution -- 1000 ml in vol.)

H_2SO_4 (Sp. gr. 1.83)	0.50 ml
$MnSO_4 \cdot 4H_2O$	3.00 g
$ZnSO_4 \cdot 7H_2O$	0.50 g
H_3BO_3	0.50 g
$CuSO_4 \cdot 5H_2O$	0.025 g
$Na_2MoO_4 \cdot 2H_2O$	0.025 g

Solution C. (Stock solution -- 1000 ml in vol.)

$FeC_6O_5H_7 \cdot 5H_2O$	10.00 g

Solution D. (Stock solution -- 1000 ml in vol.)

Glycine	3.00 g
Thiamine . HCl	0.10 g
Pyridoxine	0.01 g

Solution E. (Stock solution -- 1000 ml in vol.)

2,4-D	20.00 g
-NAA	0.10 g
Yeast Extract	0.50 g
Sucrose	20.00 g
Agar	8.50 g
Distilled water	to 1000.00 ml

One ml each of solution B, D, and E; and 2 ml of solution C were added to solution A, then the last 3 ingredients were added with sufficient water to make a total volume of 1000 ml of medium.

Strawberry plants used in the experiment were 6-week old seedlings (Sparkle--open pollinated). They were grown on sterilized soil in 2-inch pots, watered daily with distilled water and occasionally fertilized with 16-32-16 soluble fertilizer. Vigorous plants of uniform size were selected. They were removed from the 2-inch pots and the roots were washed and checked for uniformity before transplanting.

Plants were divided into 2 groups: 1 with normal root system and the other with roots pruned to about 1½ inch long. Each group was subdivided into 3 treatments, i.e., with the addition of 2000, 500 and no nematodes per pot. The nematode inocula were prepared by comminuting the clover callus tissues in 100 ml of sterilized distilled water for 10 seconds in a Waring Blendor. The resulting mixture was thoroughly agitated to insure a uniform distribution of the nematodes, and the number of nematodes in the aliquot was determined by counting a few 1-ml samples. Dilution of the aliquot was made to obtain the proper number of nematodes in a given volume, so that it could be poured into the pot before plants were transplanted. All treatments of this test were replicated 10 times.

Resulting data of both greenhouse experiments were recorded 6 weeks after inoculation.

The test tube technique herein described for testing pathogenic effects of fungi on strawberry roots was further modified. It proved to be a useful tool in testing the effect of P. penetrans on the development of strawberry root discoloration under conditions where no other soil microorganisms were present. Strawberry seedlings were grown on a nutrient agar medium in test tubes. A pure culture of P. penetrans reared on clover callus tissues was introduced aseptically into the tubes of seedlings. Various numbers of nematodes were inoculated to seedlings of different ages. Nematode-free callus tissues were added to the seedlings which served as check plants. Macroscopic and microscopic observations were made daily. The progress of pathogenicity of P. penetrans on strawberry roots was micro-photographed directly through the tubes without disturbing either the parasites or the hosts.

Results.--No difference was found in the number of meadow nematodes recovered from roots of Premier and Sparkle cultivar collected in the same field on the same sampling date. Populations were much greater at Londonderry than at Durham throughout the year. Figures 2 and 3 illustrate the seasonal fluctuation of P. penetrans in and about the strawberry roots from the 2 fields investigated. Nematode populations reached a peak in late August and early September and decreased in October and November. Nematode recovery from strawberry roots was the lowest in the early spring,

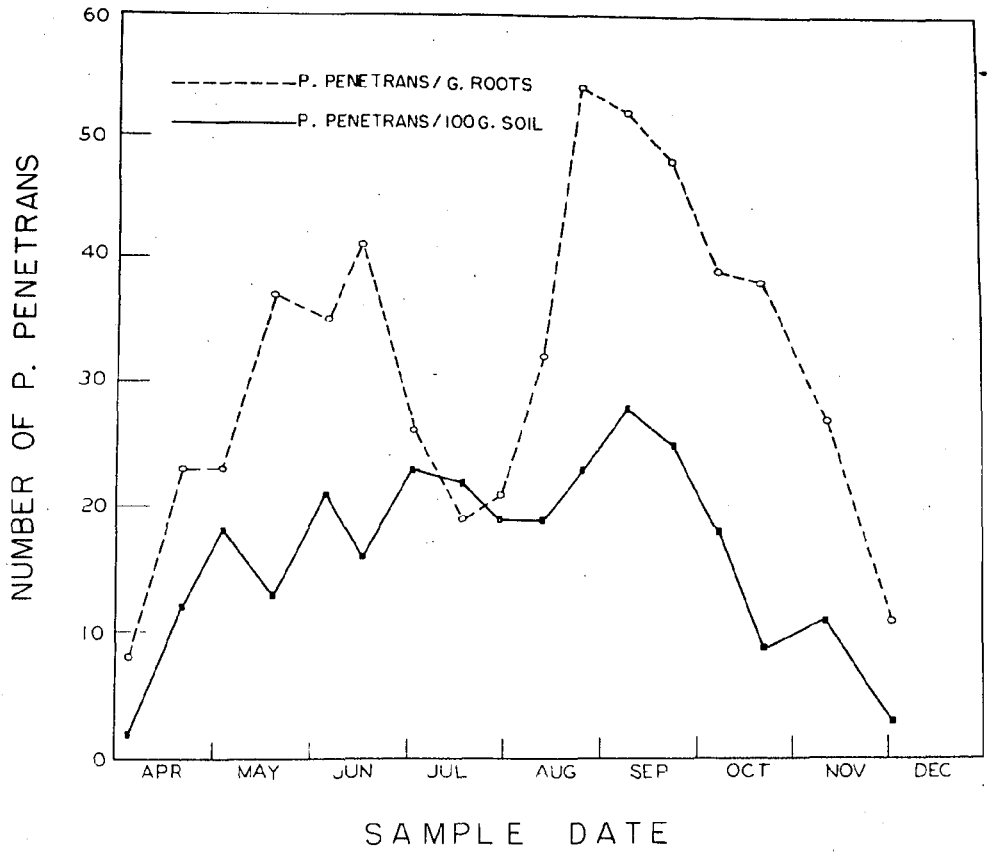

Figure 2. - Nematode population counts for strawberry roots and adjacent soil samples from Durham field plot, April to December, 1960.

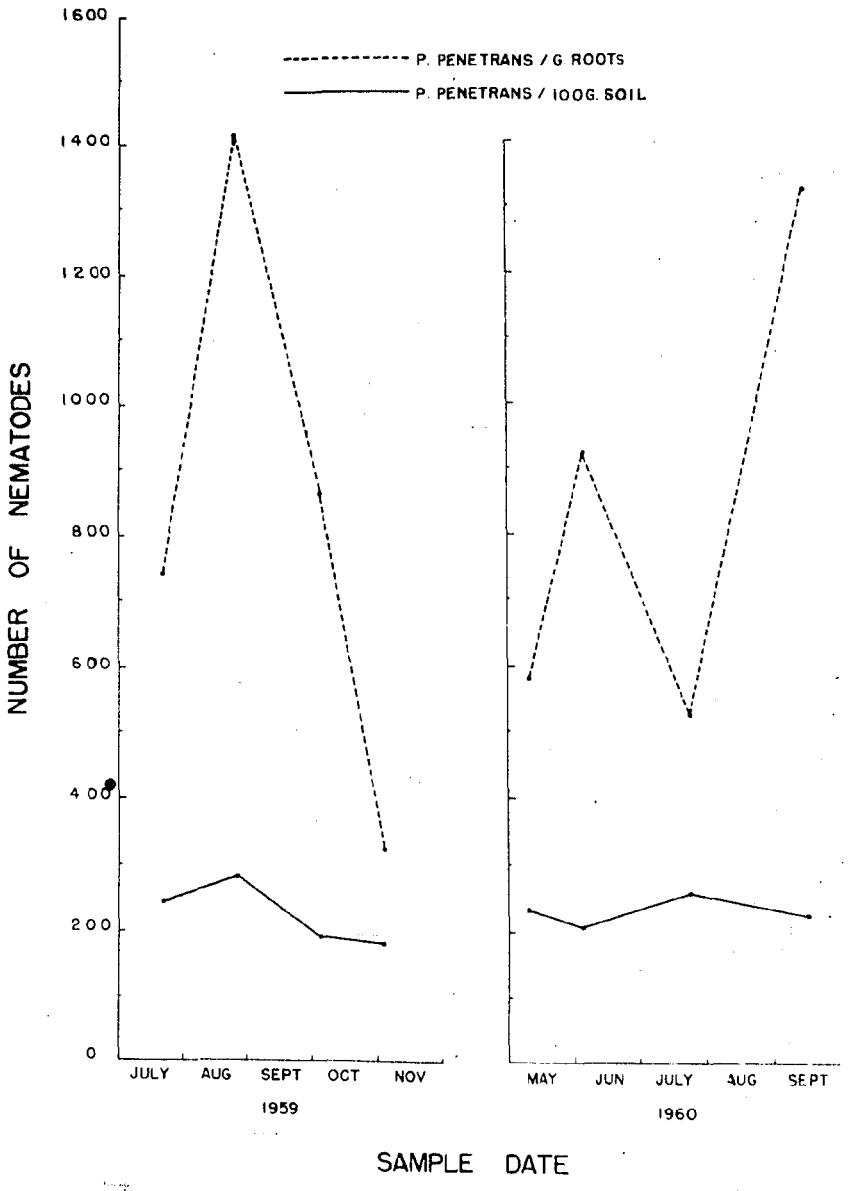

Figure 3. - Nematode population counts for strawberry roots and adjacent soil samples from a commercial field plot in Londonderry, New Hampshire, 1959-1960.

then it gradually increased during the spring and early summer. Shortly after strawberry fruiting the nematode population dropped sharply but built up again rather quickly in August. The formation of new roots in the early fall and spring probably accounts for the rise in population, while the formation of corky roots after strawberry fruiting might explain the sharp decrease in nematode populations. This might also explain why nematode numbers in soil ajacent to strawberry roots fluctuated less throughout the growing season than it did in the roots.

When pea roots infested with nematodes were used as inocula, no differences in the average root rot rating could be detected between treatments at the end of 6 weeks in the greenhouse experiments. As given in Table 11, there was also little or no difference in fresh root weights between the 2 treatments in which 2000 and 1000 nematodes were added. However, as shown by the data, a highly significant difference occurred in the average fresh root weight between the check plants and both of the nematode-infested plants. Since pea roots were mixed in the soil in this experiment, it was impossible to be certain that there were no plant parasitic fungi involved. Plating the strawberry roots from this test yielded a number of fungi. These fungi may account for the discoloration of the roots in all treatments.

The results from the second greenhouse experiment showed that P. penetrans would attack newly initiated strawberry roots more readily than old roots and they would also

Table 11. - Effect of meadow nematodes (P. penetrans) on the average fresh root weights and root rot ratings of strawberry plants grown in the greenhouse for 6 weeks.

Nematodes added	Average fresh root wt. (g) a/	Average root rot rating a/	No. nematodes per gram root	No. fungi isolated (20 sections)
2000 nema	4.05	0.8	224	18
1000 nema	4.51	0.7	89	22
No nema (check)	5.52	0.7	0	14
L.S.D. 5%	0.76			
L.S.D. 1%	1.00			

a/ Each figure represents an average of 10 plants.

inhibit root growth more severely. Table 12 shows that after roots were mechanically damaged (trimmed short) the average fresh root weight of the plants in which 500 nematodes had been added was significantly greater than those when 2000 nematodes were added. The absence of P. penetrans (check plants) resulted in a highly significant increase in root growth over both groups of nematode-treated plants. The addition of 500 or 2000 P. penetrans to the relatively older roots (unpruned) did not appear to influence their growth although it should be expected that soil under heavily infested field conditions may support much greater nematode populations than were used in this experiment. Here again, root rot ratings among the treatments showed no significant difference. It was thus concluded that even though the nematodes used were obtained from aseptic pure cultures, fungal contamination of the soil during test periods was inevitable.

The test tube technique for testing pathogenicity of P. penetrans to strawberry roots was designed to eliminate the confusion occurring in greenhouse experiments because of fungus contamination. In test tubes, nematode movement through the agar medium, accumulation around the root tips, feeding habits, and the host responses could be observed directly under a dissecting microscope (X 45) or regular microscope (X 100). P. penetrans not only produced spotted lesions on roots but also caused the entire root to turn black if a sufficient number of nematodes was present. In

Table 12. - Effect of meadow nematodes (P. penetrans) on the average fresh root weight and black root rot rating of root-pruned and unpruned strawberry plants grown in the greenhouse for 6 weeks.

Treatment and nematodes added	Average fresh root wt. (g) a/	Average root rot rating a/	No. nematodes per gram root	No. fungi isolated from 20 sections
Unpruned roots				
2000 nema	5.12	0.7	90	15
500 nema	5.36	0.4	71	18
No nema	5.60	0.4	0	8
Pruned roots				
2000 nema	2.80	0.5	195	19
500 nema	3.45	0.8	91	21
No nema	4.23	0.5	0	11
L.S.D. 5%	0.54			
L.S.D. 1%	0.72			

a/ Each figure represents an average of 10 plants.

the absence of fungi or bacteria, the pathogenicity of P. penetrans on strawberry roots under such conditions was definitely established. Figure 4 illustrates the aerial symptoms on strawberry seedlings inoculated with nematodes compared with an uninoculated check plant. There was a correlation between the limited growth of the roots due to nematodes and the stunting of the shoots. Figure 5 shows that the black rot of the roots can be caused by nematodes alone without interactions of other microorganisms. This phenomenon could be demonstrated also by inoculating strawberry seedlings with any one of the isolated TPF, as discussed before, although fungi usually produced discoloration on roots in a shorter time. Figures 6-10 show the progress of P. penetrans attacking the roots. It was found that nematodes were attracted to the longest root tip and colonized around the tip within 30 hours after inoculation. They fed on root hairs as well as epidermal cells (Figure 11). Some nematodes entered the roots but the majority stayed outside under such conditions. Eggs were laid along the roots where nematodes were feeding and their hatching process could be easily observed under a microscope. Root tissue responded to the nematode attack usually with hypertrophic growth of cells, and gradually became necrotic. If only a few nematodes were present, i.e., 1-10 nematodes per tube, the roots would continue to grow or initiate branching rootlets and overcome any damage done by the nematodes. If a large number of nematodes were present the root tip

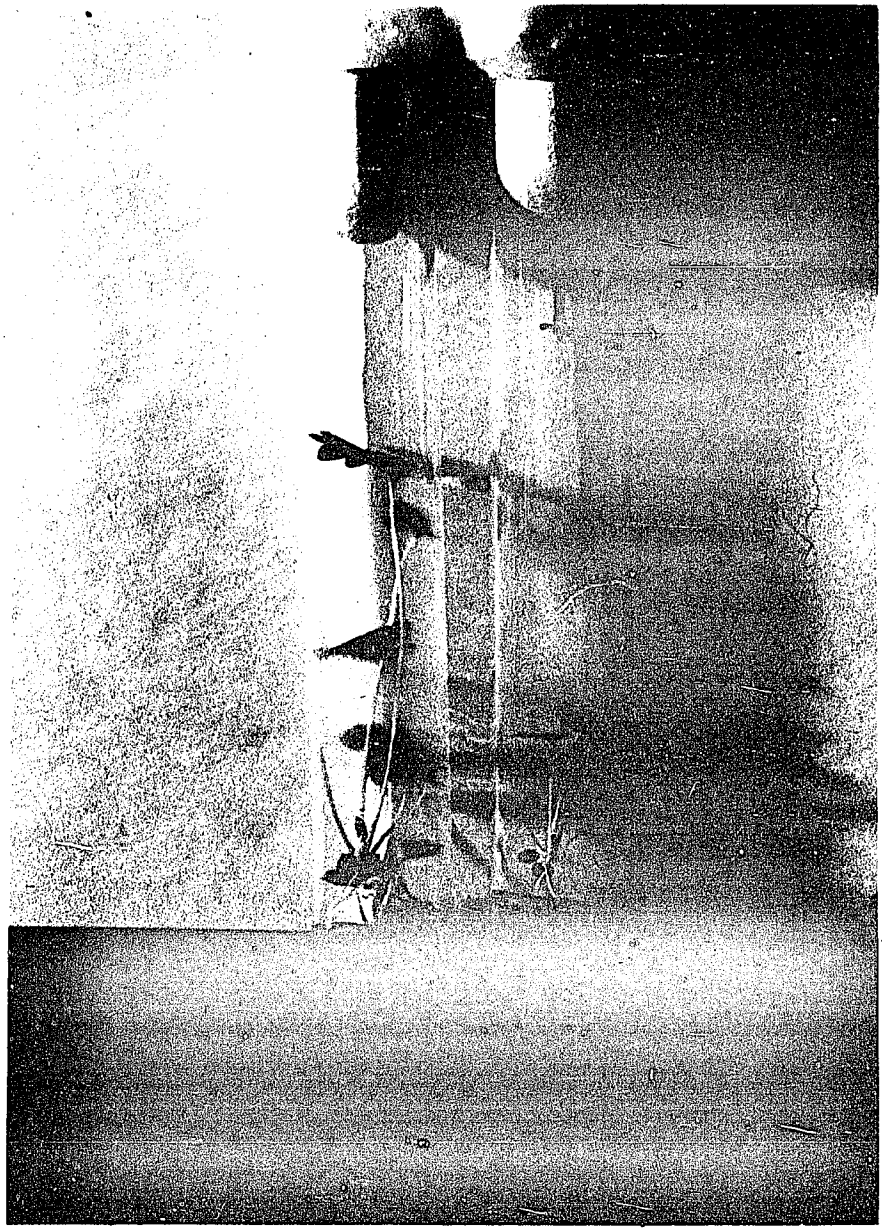

Figure 4. - Test tube technique used for testing pathogenicity of P. penetrans on strawberry seedlings. Seedling on left uninoculated. Note the difference in shoot and root growth 6 weeks after inoculation.

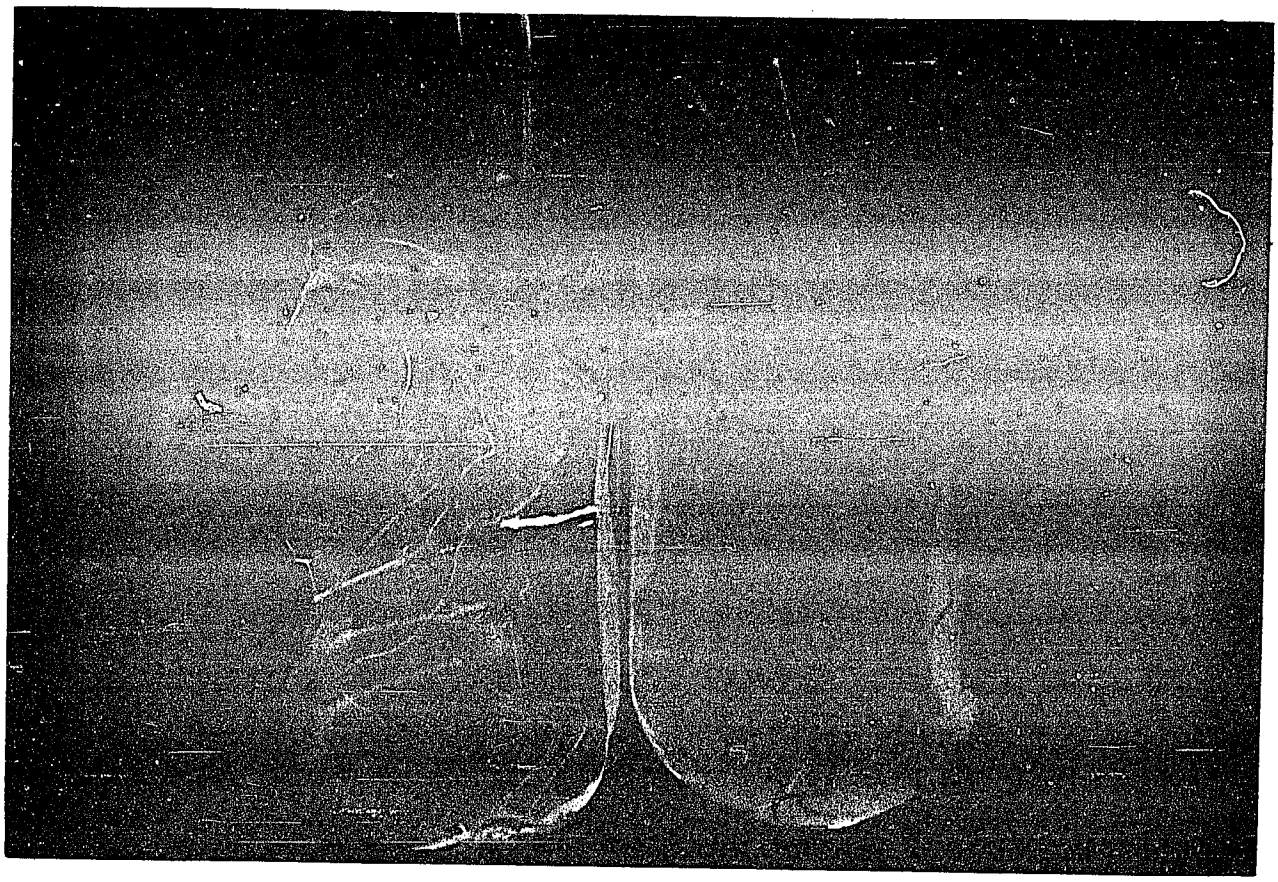

Figure 5. - Effect of P. penetrans on the production of black root rot of strawberry seedlings grown in test tubes. Uninoculated control, left; nematode-infested tube, right. The photograph was taken 2½ months after inoculation.

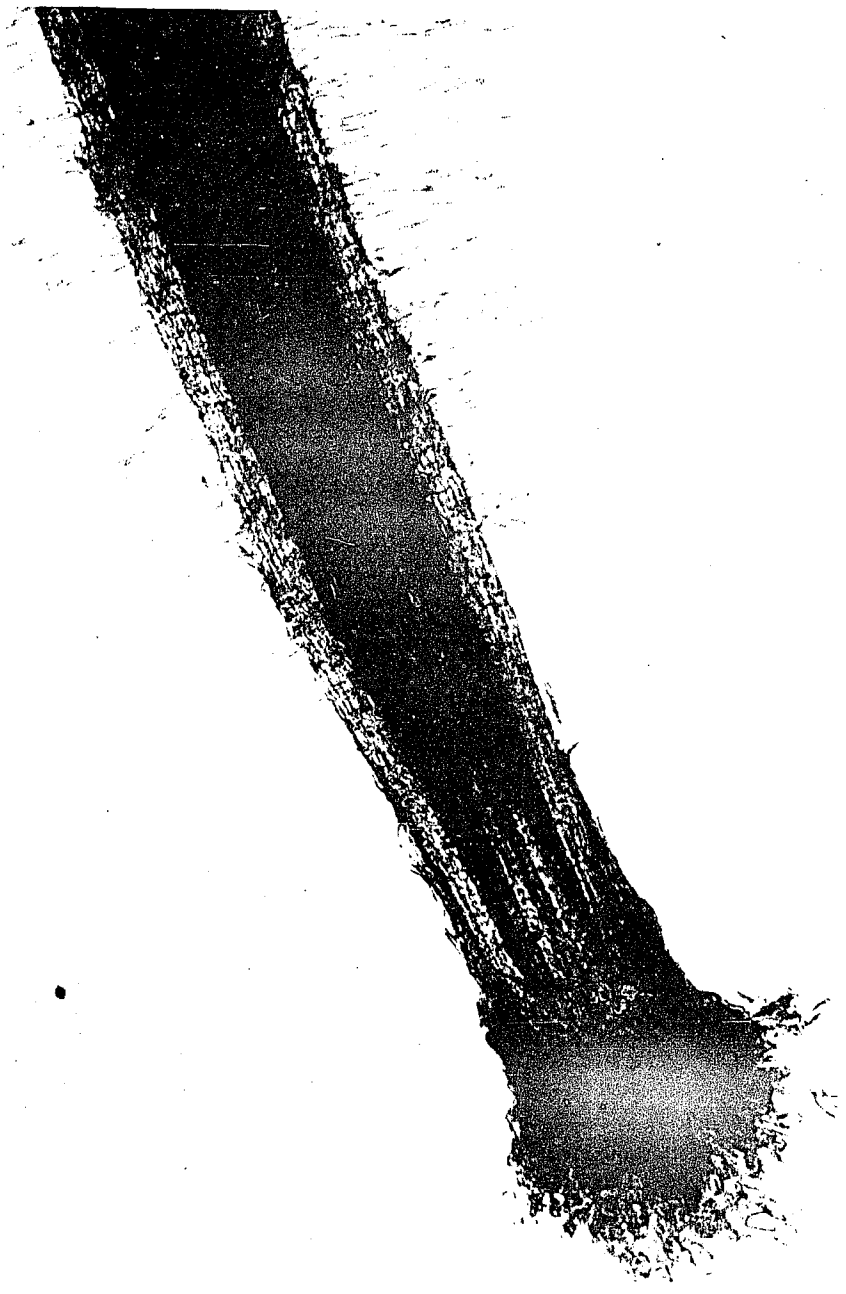

Figure 6. - Root tip of a healthy strawberry seedling grown on nutrient medium in a test tube. Note the abundance of root hairs. (X100)

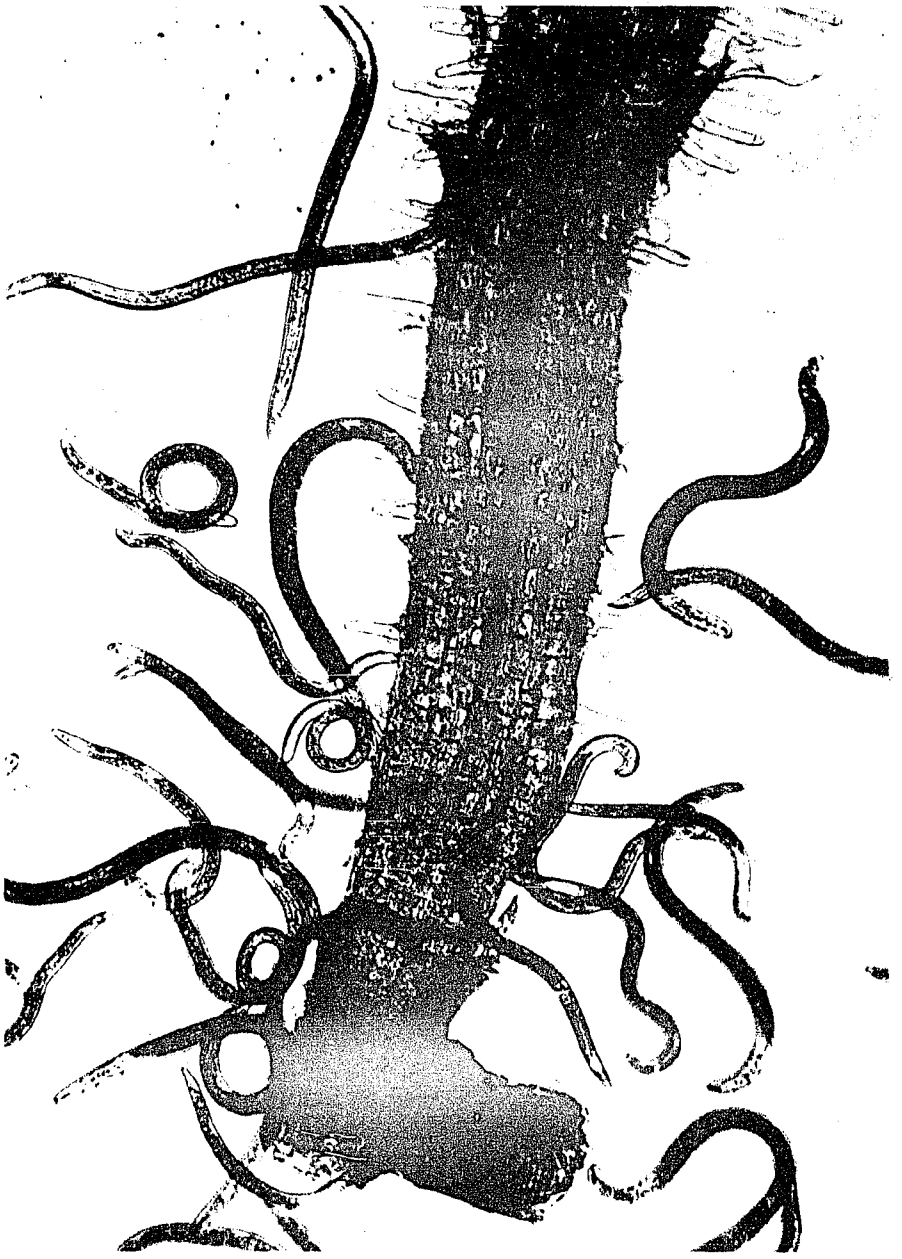

Figure 7. - Root of strawberry seedling photographed in a test tube, showing nematodes colonized around the root tip, 20 hours after inoculation.(X100)

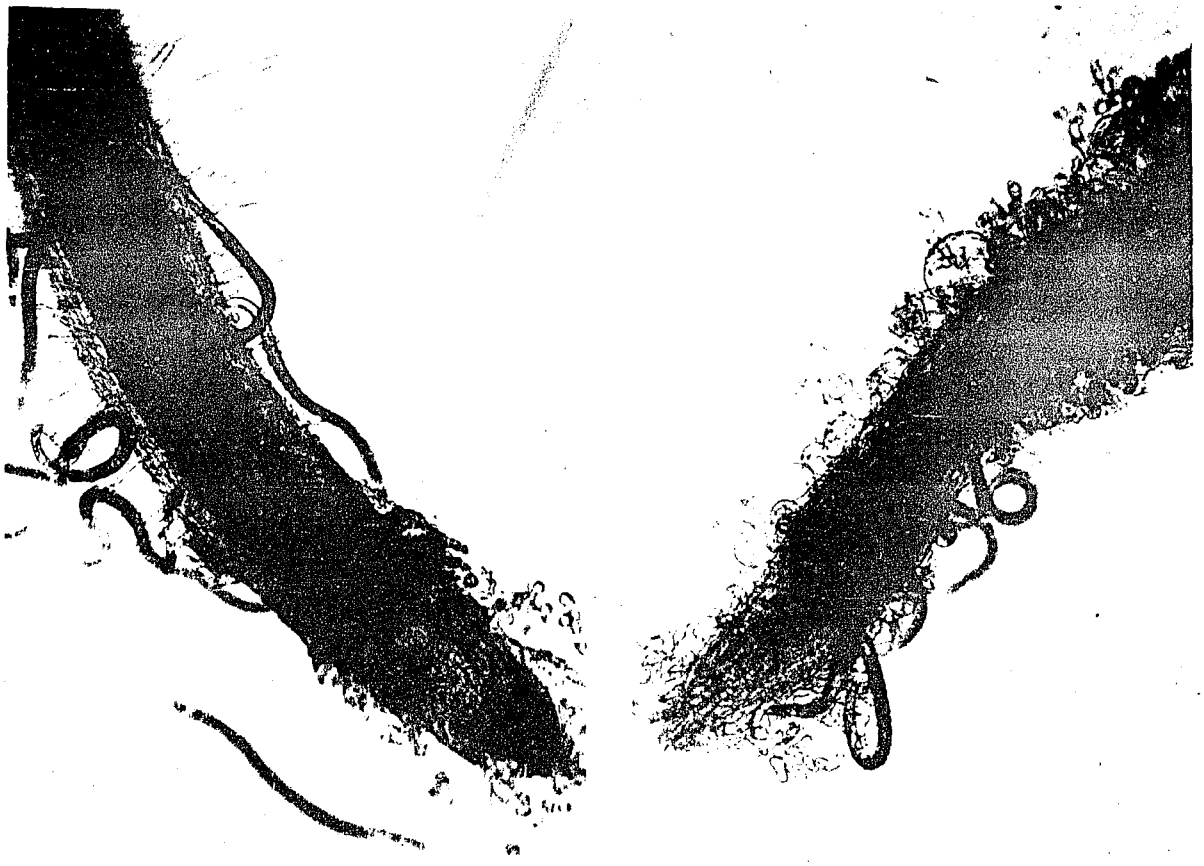

Figure 8. – Strawberry roots photographed in test tubes, showing injury caused by P. penetrans. Left: 1 week after inoculation. Right: 1 month after inoculation.(X100)

Figure 9. - Strawberry roots photographed in test tubes, showing injuries caused by P. penetrans 2 months after inoculation. Note the lack of root hairs at left and the swollen root tip at right. (X100)

Figure 10. - A blackened strawberry root caused by P. penetrans, 3 months after inoculation.(X100)

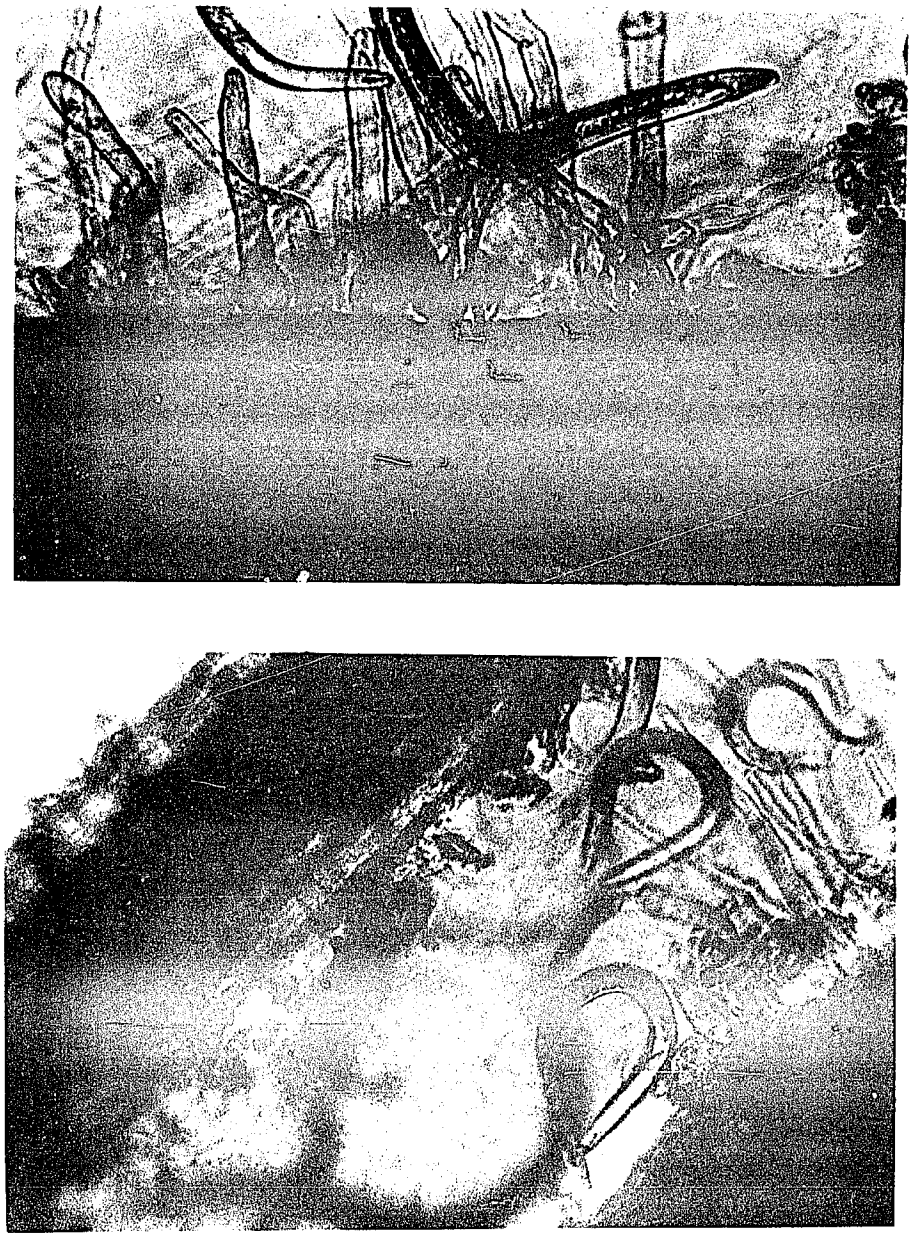

Figure 11. - Strawberry roots photographed in test tubes, showing nematodes feeding on root hairs (top) as well as on epidermal cells (bottom). (X430)

meristem was damaged and ceased to grow. Nematodes would migrate to nearby branch roots and attack the tips in the same manner. Soon all the root growth was inhibited and black rot symptoms became evident.

Section IV. Experiments Combining Fungi And Nematodes

Materials and methods.--From July 1960 to October 1961 a field plot experiment, employing a 4 x 4 Latin Square design was conducted at the University in Durham. The size of each plot was 100 ft^2 ($10' \times 10'$). Soil treatments within the experimental area were prepared as follows: (1) 4 plots were fumigated with methyl bromide at the rate of 1 pound per 100 ft^2. The plots were covered with polyethylene sheets during fumigation and remained covered for 72 hours; (2) 4 plots were fumigated with ethylene dibromide (41% actual EDB by volume) at the rate of 1 ml per ft^2, injected to a depth of 8 in. and covered with soil for 3 weeks; (3) 4 plots were fumigated with methyl bromide as described in (1) but were reinfested with approximately 40,000 meadow nematodes per plot when strawberry plants were transplanted to the plots; and (4) 4 plots with no treatments. The soil had been sampled before treatment and found to contain about 5-10 P. penetrans per gram of soil.

Strawberry plants (cultivar Premier) used in this experiment were 4-week old runner plants. They were rooted in steam-sterilized soil in 4-inch pots, a method previously described. In July 1960, the plants were removed from the pots and transplanted directly to the field plots. Two rows of 4 plants each were set in each plot. Individual plants were spaced 2 ft. apart with 5 ft. between rows to

provide ample space for runner plant growth. Only the first runner plants and the next runner developed from them were saved and they were trained in a direction approximately perpendicular to that of the rows. Fertilizer (10-10-10) at a rate of ½ lb per plant was applied 1 month after transplanting. Foliage spray with Thylate (2 lb/100 gal) was made once in the spring of 1961. Weeds were removed with a hoe or by hand.

Five samples were collected from May to October in 1961. For sampling, 8 runner plants, established in the previous year, were carefully dug from each plot, labeled and stored in polyethylene bags in a refrigerator. The root rot ratings and methods of recovering nematodes and isolating fungi from the roots were made in the same manner as described in the previous sections of this paper.

A greenhouse experiment similar to that conducted in the field was set up in June 1961. Soil was collected from the University experimental farm near the strawberry field plot. Four soil treatments were made in the greenhouse: (1) steam sterilized for 4 hr; (2) fumigated with ethylene dibromide (41% actual EDB by volume) at the rate of 1 ml per 6-inch pot of soil. After 2 weeks each pot was inoculated with 10 ml of mixed TPF suspension; (3) steam sterilized soil with the addition of 2000 P. penetrans per 6-inch pot; and (4) untreated field soil with the addition of 2000 P. penetrans plus 1 ml of mixed TPF suspension per 6-inch pot. Nematode inocula were obtained from pure cul-

tures of P. penetrans reared on clover callus tissue. Fungus inocula consisted of 1 plate of each TPF pure culture and mixed thoroughly with distilled water for 30 seconds in a Waring Blendor.

Four-week old strawberry seedlings (Sparkle--open pollinated) grown in steam-sterilized soil in 2-inch pots were transferred directly to the 6-inch pots containing soil with 1 of the soil treatments mentioned above. Each treatment was replicated 8 times. Results were determined 7 weeks later.

Results.--Severe winter weather occurred in January and February of 1961, then a late frost occurred in May which injured over 70% of the strawberry blossoms in the University experimental plots. Fortunately, all plants grown in the test block survived. Visual differences were noticed in plants grown in plots having different soil treatments. Plants grown in methyl-bromide-treated plots with or without the addition of P. penetrans were more vigorous than the others and they produced more runner plants with larger leaves and fruits. Weeds were also well controlled in those plots. Plants grown in ethylene-dibromide-treated plots showed no apparent difference in growth as compared with those plants grown in the untreated check plots.

The first plant sample was taken in May. Roots in all plots developed well. Winter injury was observed occasionally on portions of roots close to the crowns. No

differences could be detected in the degree of root rots among all treatments, and a statistical analysis of the data showed no significant difference in root rot ratings between the treatments for a given sampling date. However, there were striking differences in degree of rot from plants taken in the same treatment on different sampling dates. The results from 5 samples are summarized in Table 13. The data, subjected to analysis of variance, shows that the differences of average root rot ratings throughout the growing season in 1961 could be attributed to different treatments. After 15 months of plant growth, methyl bromide had controlled root rot enough to be highly significant over the untreated plots. Even with the addition of nematodes, the rot rating was still significantly lower than in the plants from the ethylene dibromide treated plots. It indicated that either 40,000 nematodes per plot did not provide sufficient inoculum to produce root injury or it was good evidence that fungi probably still play a major role in the development of black root rot. There seemed to be no difference in the control of nematodes among the various treatments although contamination in the field under conditions of this experiment could be expected. Table 13 shows that a small number of P. penetrans associated with strawberry roots was recovered from plants from every treatment. Therefore, differences in fresh root weight among treatments probably were not due to nematode control.

The greenhouse test was quite similar to the field

Table 13. - Effect of soil treatment on the development of black root rot of strawberry plants grown in Durham field plots, 1960-1961.

Treatment	Average root rot rating				Average root rot rating at end of the growing season	Average fresh root weight in grams[a]			
	5/29	7/5	8/4	10/15		5/29	7/5	8/4	10/15
Methyl Bromide	0.36	1.91	2.45	2.60	1.83	10.38	8.92	8.68	8.19
EDB	0.61	2.85	3.31	2.98	2.44	8.40	6.61	6.95	5.97
MB + nematodes	0.43	2.40	2.31	2.88	2.01	10.38	8.87	8.65	8.05
Untreated	0.72	3.00	3.66	3.07	2.61	9.05	7.43	6.70	7.51
					L.S.D. 5% 0.32				
					L.S.D. 1% 0.43				

(Table 13 continued on next page)

Table 13. - Continued.

Treatment	No. *Pratylenchus* spp. recovered per gram roots				Fungi isolated from 80 root sections plated							
					5/29		7/8		8/4		10/15	
	5/29	7/5	8/4	10/15	No. isolated	% of TPF [b]	No. isolated	% of TPF	No. isolated	% of TPF	No. isolated	% of TPF
M B	6	19	40	34	63	83	76	78	76	63	71	73
EDB	5	13	40	46	76	90	96	72	73	58	93	66
MB + nemas	8	33	45	78	72	85	83	63	70	59	87	79
Untreated	10	59	62	54	92	89	94	57	94	61	99	59

[a] Each figure represents an average of 32 plants from 4 plots sampled in 1961.

[b] Based on the results obtained from fungus pathogenicity tests in test tubes.

test, except that larger quantities of both nematodes and fungi were added to the greenhouse soil. The results are presented in Table 14. Statistical analysis showed that variation of fresh root weight among treatments was entirely due to nematode injury whereas variation in root rot rating among treatments was a result of fungal invasion. No interaction of fungi and nematodes appeared to exist. In the absence of P. penetrans in the soil a highly significant increase in root growth was obtained in uninoculated check plants, and, in the absence of TPF, root rot decreased significantly.

Examination of the strawberry roots taken from soils where both fungi and nematodes were added showed that there were no nematodes in the rotted portions of the roots. This explains the fact that fewer nematodes were recovered from plants in the soil treated with fungi plus nematodes than in the soil treated with nematodes alone.

Attempts were made also to test the interaction of individual TPF with P. penetrans in test tubes where strawberry seedlings were grown on agar medium. TPF Nos. 8, 9, 16, and 82 were tried without success, because when a fungus established itself on the roots, nematodes were either killed or moved away from the roots. However, fungus mycelium was observed to establish itself more readily on the necrotic tissues previously damaged by nematodes than on healthy tissues. It indicated that these TPF were primarily facultative parasites and that P. penetrans could open an avenue on strawberry roots for fungal invasion.

Table 14. - Effect of meadow nematodes (P. penetrans) and fungi on the average fresh root weight and black root rot rating of strawberry plants grown in the greenhouse after 7 weeks.

Treatment	Avg. fresh root wt. (g)[a]	Avg. root rot rating[a]	No. nemas recovered per root	Fungi Isolation Sections plated	No. TPF	% of TPF[b]
Check	9.93	0.88	0	20	11	18
2000 nematodes added	6.49	1.63	182	20	9	11
10 ml mixed TPF added	8.18	2.13	3	20	23	83
nemas and TPF added	6.03	2.00	123	20	30	60
L.S.D. 5%	2.03	5% 1.05				
L.S.D. 1%	2.74	1% 1.42				

[a] Each figure represents an average of 8 plants.

[b] TPF = test-tube-tested pathogenic fungi.

DISCUSSION AND CONCLUSIONS

Black root rot was found to be a common and sometimes a serious disease of strawberries in New Hampshire. A strawberry plantation affected with root rot may survive for 2 or 3 years and produce at least one fairly satisfactory crop. In some cases, however, strawberries planted in the spring were so "patchy" or weak by fall that the plantations were abandoned. A strawberry plant may withstand some loss of its root system with no detrimental effects provided the environmental conditions are favorable for plant growth.

This study clearly demonstrates that strawberry roots provide tenancy for numerous alien organisms, parasites as well as saprophytes. From lesioned strawberry roots, a total of 107 fungal types representing at least 96 fungal genera were isolated. Nematodes, especially Pratylenchus penetrans, were found consistently associated with strawberry roots grown in all soil types at any time of the year. Therefore, all the rhizospheric inhabitants must be given serious consideration in connection with the development of black root rot.

The rhizospheric microorganisms are dependent largely upon roots of specific plants for their growth. Below ground, we deal frequently not with straightforward parasite-host disease relationships as in above-ground pathology, but with the still somewhat unconventional pathology where

the "outsiders" as well as recognized invaders may be involved in a disease complex. Root parasitism by weak parasites is usually favored by factors of the above and below-ground environment which favor the parasites more than the host. Furthermore, an interaction of organisms may occur in the case of black root rot. This is reflected in the fact that a large number and diversity of fungi were isolated. Many of them, unable to produce remarkable disease symptoms under favorable conditions, probably live primarily by exploiting the infected or injured roots. Therefore, various attempts were made to study the relationship existing between each associated organism and its host, so that valid conclusions could be reached when only the host and one specific parasite interacted. The test tube technique proved to be successful. It allowed us to apply one unknown variable at a time, or in other words, the effect of one specific fungus isolate or one species of nematode could be studied. Consequently, 27 different fungi and P. penetrans have proven to be pathogenic to strawberry roots.

The 27 fungal isolates found to be pathogenic under test tube conditions (TPF) varied in their capacity to penetrate and infect living root tissues. They comprised over 70% of the total fungal isolates. However, those TPF which were most frequently encountered did not always coincide with their relative pathogenicity. Under greenhouse or semi-natural conditions all TPF cultures could produce rot on artificially weakened strawberry roots and could be reisolated

readily, but they showed less effect on established root systems. Accumulative evidence has led us to believe that TPF were inhabiting the rhizosphere as facultative parasites. During their parasitism on roots, they were cortical parasites and could be highly injurious to the feeder rootlets. Once a portion of the root system was attacked and rotted, the rotted roots would then harbor a high concentration of fungi and be a constant threat to the neighboring healthy roots.

The strawberry is a perennial plant. The new root growth may occur during part or all of the dormant season when the shoot has low demands on food. The 2 peaks of root production in this area are in early spring and following harvest. Any potential parasite in the rhizosphere could initiate a successful primary invasion on roots at these periods. In this study, patches of rot lesions were often observed on the new roots shortly after their formation. Later in the spring, as the shoot is expanding and blossoms are developing, roots cannot compete for food, and growth stops. The secondary invaders, which may be saprophytes or any TPF, may effect displacement of the primary parasite from the original invaded site, or the initial parasite may still maintain its spatial position. Therefore, the invasion of these parasites or pathogens must be capable of destroying a large percentage of new roots and feeder rootlets and thus must result in weak and sick plants. Repeated examination of strawberry plants when collecting root samples

throughout the years during the preliminary survey and during experimental processes showed that this was actually the case. Root rot disease ratings increased from spring up to fruit harvest time, but after this time visible injury was greatly reduced. Although a large number of different types of fungi were isolated in the summer months, a relatively lower percentage of TPF was obtained in summer than in the spring or fall. It is evident that in root rot attack a continual struggle is going on between the plant and the rhizospheric inhabitants.

Since P. penetrans was found consistently associated with strawberry roots, the role that nematodes play in the development of black root rot was studied. Field investigations showed that populations of P. penetrans in and about strawberry roots fluctuated throughout the growing season. Their number showed a steady increase in the spring and a sharp drop shortly after fruiting, but populations rebuilt quickly in late August. The peak of population was reached in August and September and then decreased in October and November. The formation of new roots in fall and early spring probably accounts for the rise in population while the formation of corky roots after fruiting might explain the sharp decrease in number. These facts can explain also why nematode populations in soil adjacent to roots fluctuated less than inside the roots.

In the laboratory, a pure culture of P. penetrans was observed to produce typical black root symptoms on roots

of strawberries grown in test tubes, although the progress of discoloration was much slower than when TPF were used as inocula. Thus far, the experiment not only verified some early reports that meadow nematodes were primary parasites of strawberry but also proved the pathogenicity of this nematode to strawberry roots in the absence of other microorganisms. Under natural conditions, even though it should be expected that soil of heavily infested fields may support greater densities of nematode population than in greenhouse or test-tube tests, it is unlikely that P. penetrans would be solely responsible for the incidence of the black root rot. However, they are at least involved in the initial phase of the development of the disease.

In greenhouse as well as test-tube experiments, very few, if any, nematodes could be found in the rotted regions where fungi have become established. It was observed that fungi usually infected roots more readily at the necrotic portions previously injured by nematodes than they did healthy tissues. It indicated that P. penetrans can efficiently provide an opening site for TPF invasion. The synergistic effects on the development of black root rot probably exist only at the initial phase of the infection. In addition, according to the greenhouse experiments with both fungi and nematodes, variation of fresh root weight among treatments was entirely due to nematode injury whereas variation in root rot rating among treatments was a result of fungal infection. Thus, it further demonstrated that there

was no interaction of fungi and nematodes during the progress of the rot. On the other hand, the reduction of fresh root weight or the continuous root pruning by nematodes plus progress of root rot by fungi did show the effect on plant vigor. It is doubtless safe to say that both parasites must play a complementary effect on the net result of weakening the plant.

It is difficult to try all possible combinations of all the fungal isolates with or without the addition of P. penetrans to find the most virulent group in the development of the rot and draw a definite conclusion as might be done with a single-pathogen disease. Which would cause the greatest damage to the strawberry roots, the continued action of the nematodes unhindered by fungi or the action of the fungi unaided by the nematodes? Both TPF and P. penetrans were primary parasites of proven pathogenicity. Large numbers of secondary invaders probably played some minor roles also. Therefore, it is obvious that many facets of the intricate system of interactions between plant, rhizospheric microflora and parasites need to be explored.

Unfortunately, field experiments gave unconvincing results due to difficulties of controlling many variable factors. Methyl bromide only temporarily suppressed parasites in the soil but it still provided relatively better plant growth and reduced root rot over a period of 15 months. Plants probably became well established in this soil before the soil was contaminated by parasites and their action on

the roots had taken place. Better plant growth of both above and below-ground parts in methyl bromide-treated soil was also partially attributable to weed control. Soil fumigated with ethylene dibromide at a rate of 1 ml per 1 ft^2 had no apparent effect in controlling black root rot. There was no evidence of markedly affecting nematode population or of improving plant growth. TPF were isolated from strawberry roots grown in different soil treatments, and nematodes appeared in very small numbers. Therefore, it again suggested that fungi played an important role in the entire development of the rot while P. penetrans was involved only in the beginning stages of infection.

SUMMARY

Preliminary investigations in 1958 and 1959 showed that strawberry black root rot is a common disease in New Hampshire. Studying samples of strawberry roots clearly revealed that they harbored numerous alien organisms. In 1959 and 1960, root samples were obtained periodically during the growing seasons from 7 selected field plots. From these roots, a total of 3865 fungal isolates were obtained. They were grouped into 107 fungal types representing at least 96 genera. In addition, Pratylenchus penetrans, the predominant form of meadow nematodes, was found consistently associated with strawberry roots. P. penetrans was also recovered from roots of many wild and cultivated plants grown near or in strawberry fields. Techniques used in connection with isolation and recovery of fungi and nematodes are described.

Various attempts were made to study the relationship existing between each isolated organism and the host roots in the development of black root. A test-tube technique was developed which proved to be successful. As a result of inoculation studies, 27 fungi and P. penetrans were shown to be pathogenic on strawberry roots.

The 27 fungi found to be pathogenic in test tube tests (TPF) comprised over 70% of the total fungal isolates, although they differed greatly in their relative prevalence. However, those TPF which were most frequently encountered

did not always coincide with their relative pathogenicity. Further greenhouse experiments showed that all TPF cultures could cause lesions on weakened strawberry roots. Accumulating results led us to believe that TPF are inhabiting the rhizosphere as facultative parasites, and they live primarily by exploiting the weakened or injured roots.

P. penetrans was cultured aseptically on clover callus tissue. These nematodes produced typical black root symptoms on roots of strawberry seedlings grown in test tubes. The progress of root discoloration caused by nematodes was slower than when TPF were used as inocula. However, it was proven that P. penetrans, in the absence of other microorganisms, also was pathogenic to strawberry roots.

Nematode populations in and about strawberry roots were studied. Populations in roots fluctuate greatly throughout the growing season and reach a peak in late August and early September. Populations in soil adjacent to roots fluctuate much less than inside the roots. The formation of new root systems in the spring and fall probably accounts for the rise of nematode numbers.

The role of TPF and P. penetrans in the strawberry black root rot complex was studied in field and greenhouse experiments. Results obtained from both experiments indicated that there was no continuing interaction between fungi and nematodes in the development of black roots. Nematodes usually moved away from roots when the roots became discolored or were invaded by fungi. Under test tube conditions,

fungi usually infected roots more readily at the necrotic portions previously injured by nematodes than they did healthy tissues. Therefore, all the evidence suggests that P. penetrans can provide an opening site for fungal invasion. The synergistic effects on the development of black root rot between P. penetrans and TPF probably exists only in the beginning stages of the infection. TPF and the other secondary soil fungi still carry the important role in the progress of the rot.

It is contended that the black root rotting plus the constant root pruning caused by the actions of pathogenic fungi (TPF) and Pratylenchus penetrans as described herein are the main factors causing an adverse effect on the growth of strawberry plants.

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APPENDIX

Identification of fungi which were pathogenic to strawberry roots in test-tube tests.

Assigned TPF No.	Fungus Identification	Identified by
3	_____[a]	
6	*Phialophera* sp.	S.J. Hughes
8	*Trichoderma* sp.	T.A. Chen
9	_____[a]	
11	_____[a]	
12	*Paecilomyces* sp.	S.J. Hughes
13	_____[a]	
18	*Rhizoctonia* sp.	T.A. Chen
20	_____[a]	
25	_____[a]	
30	_____[a]	
34	*Penicillium* sp.	T.A. Chen
41	_____[a]	
42	_____[a]	
47	_____[a]	
48	*Phoma* sp.	T.A. Chen
55	_____[a]	
56	_____[a]	
62	*Synsporium biguttulatum* Preuss	M.A. Rosinski
70	*Coniothyrium fuckelii* Sacc.	M.A. Rosinski
77	*Gliocladium fimbriatum* Gilman & Abbott	M.A. Rosinski
79	_____[a]	
82	*Fusarium oxysporum* Schlect	R.A. Kilpatrick
84	*Alternaria* sp.	T.A. Chen
85	*Fusarium roseum* Link	R.A. Kilpatrick

Identification of fungi which were pathogenic to strawberry roots in test-tube tests. (Cont.)

Assigned TPF No.	Fungus Identification	Identified by
86	*Trichocladium asperium* Harz	S.J. Hughes
87	_____[a]	

[a] Unidentified fungus.