THE EFFECTS OF THE NEMATODE *APHELENCHUS AVENAE* ON THE DAMPING-OFF DISEASE OF PEA

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

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Macdonald College
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Montreal, Quebec

July 1978

Suggested short title

APHELENCHUS AVENAE AND DAMPING-OFF OF PEA

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ABSTRACT

M.Sc. CHOO PENG HONG Plant Science

THE EFFECTS OF THE NEMATODE APHELENCHUS AVENAE
ON THE DAMPING-OFF DISEASE OF PEA

The fungus Pythium ultimum was isolated from local soil and found to cause pre- and postemergence damping-off of pea seedlings (Pisum sativum L.).

The mycophagous nematode, Aphelenchus avenae was found to feed and multiply on P. ultimum, but not on pea seedlings when tested in vitro and in sterilized soil.

When different numbers ($25x10^3$ - $100x10^3$) of A. avenae were added simultaneously with P. ultimum to determine the effect of the nematodes on pre- and postemergence damping-off of pea seedlings, the emergence and survival percentage of the pea seedlings in sterilized soil was between 33 and 86%. When P. ultimum was the only inoculum, this percentage was down to between 0 and 26%. In unsterilized soils, with $50x10^3$ to $100x10^3$ nematodes plus P. ultimum, the emergence was between 46 and 73%, whereas with the fungus alone, this was reduced to 13%. The addition of $75x10^3$ A. avenae gave slightly better emergence of pea seedlings from soil infested with P. ultimum than did double the recommended concentration of a fungicide containing 2.5% Oxine Benzoate.

RESUME

M.Sc. · CHOO PENG HONG Plant Science

LES EFFETS DU NEMATODE APHELENCHUS AVENAE SUR LA FONTE DES SEMIS DU POIS

Le cryptogame Pythium ultimum isolé du sol régional cause la fonte des semis du pois (Pisum sativum L.) en pré- et en post-émergence.

Le nématode mycophage Aphelenchus avenae se nourrit de P. ultimum, tout en se reproduisant, mais non de plantules de pois dans des essais in vitro sur sol stérilisé.

Si de 25 x 10^3 à 100 x 10^3 individus d'A. avenae sont ajoutés simultanément avec P. ultimum à du sol stérilisé, afin de déterminer l'effet des nématodes sur la fonte des semis, le pourcentage d'émergence et de survie est de 33 à 86%. Si P. ultimum est le seul inoculum, ce pourcentage n'est plus que de 0 à 26%. Dans des sols non-stérilisés auxquels sont incorporés de 50 x 10^3 à 100 x 10^3 nématodes et du P. ultimum, l'émergence est entre 46 et 73%, tandis que pour le cryptogame seul, elle n'est que de 13%. L'addition de 75 x 10^3 A. avenae à un sol infesté de P. ultimum donne une émergence légèrement supérieure à celle obtenue avec un fongicide (contenant 2.5% benzoate d'oxine), utilisé à une concentration double de celle recommandée.

ACKNOWLEDGEMENTS

The author would like to express his sincere appreciation for the valuable guidance and constructive criticism freely given by his research supervisor, Professor R. H. Estey, during the work presented here and also during the preparation of this manuscript. He would also like to thank Professor R. L. Pelletier for his helpful advice on certain problems and for his translation of the abstract, and to Professor M. A. Fanous for his valuable assistance in the analysis of the data. Thanks are also due to others who gave many helpful suggestions.

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I. INTRODUCTION

The pea, Pisum sativum L., is a relatively cool temperature crop (optimum 24°C) that is cultivated throughout the world where cool temperature prevails during the growing season. In some respects the pea may be considered a sensitive crop and subject to attack by many phytopathogenic organisms. Chupp and Sherf (1960) listed 4 root diseases, 9 above ground diseases, plus 6 viruses. Conners (1967) listed 10 root diseases, 13 above ground diseases and 6 viruses of peas in Canada. Hagedorn (1973), who considered only the major pea diseases, recorded 6 root diseases, 3 above ground diseases and 8 diseases due to viruses.

The damping-off disease of seedlings is widely distributed all over the world. Preemergence and postemergence damping-off, which have for many years been a problem in the cultivation of wrinkle-seeded peas, have usually been attributed to soil-borne fungi, particularly species of Pythium, Fusarium, Aphanomyces, Rhizoctonia, and Ascochyta (Reinking, 1942; Schroeder, 1953; Flentje and Saksena, 1964; Escobar et al., 1967; Kraft and Burke, 1971, and others).

The incidence of pre- and postemergence damping-off of peas is influenced by many factors, including soil moisture, soil temperature, host exudates, cultivar, soil type, other soil flora and so on. High soil moisture and cool temperature generally favor plant parasitism by

species of Pythium (Horsfall, 1938; Hare, 1949; Angell, 1950a, 1950b; Kraft and Roberts, 1969; Short and Lacy, 1976, and others).

Nematodes of the species Aphelenchus avenae, Bastian, 1865, are primarily mycophagous. They have been found in virtually all types of soil from many parts of the world. A few workers (Steiner, 1936; Chin and Estey, 1966; Terry, 1966) have found them to have limited capabilities for parasitizing higher plants, although they have been found to feed and to multiply on aseptically cultured tissues of certain plants (Barker and Darling, 1965; Klink, 1966, and others).

There are numerous reports of the feeding and multiplication of A. avenae on phytopathogenic fungi (Hechler, 1962; Mankau and Mankau, 1963; Townshend, 1964; Chin, 1964; Evans, 1970; Kondrollochis, 1977, and others). Its potential for the control of fungal root diseases has been assessed by several workers (Rhoades and Linford, 1959; Klink and Barker, 1968; Roy, 1973; Cheng and Tu, 1974; Porth, 1975). However, there are few reports of the interaction between A. avenae and Pythium species in relation to the damping-off disease of seedlings.

The present studies were undertaken to determine the possibilities of using A. avenae for the control of seedling damping-off of peas due to species of Pythium and to compare this type of biological control with control by means of a standard chemical compound.

II. LITERATURE REVIEW

Fungal relationships of Aphelenchus avenae

Christie and Arndt (1936) reported the feeding of A. avenae on hyphae of Neurospora sitophila which involved removal of hyphal contents. Hechler (1962) described petri dish culture of A. avenae feeding on Pyrenocheata terrestris. He found that an initial colony of 100 adults of A. avenae could increase to as many as 100,000 in 14-16 days at 28°C on the tested fungus. Mankau and Mankau (1963) reported extensive population development of A. avenae on several phytopathogenic soil fungi. They also noted that neither Phytophthora spp. nor Pythium spp. were good hosts. They also observed that A. avenae did not feed on thick-walled chlamydospores, conidia, or other survival structures of fungi. Townshend (1964) found that A. avenae reproduced on 54 out of the 59 species of fungi he tested. He also recorded that three species of Botrytis supported between 50,000 and 243,000 progeny of A. avenae.

Chin (1964), who experimented with twenty species of soil fungi, reported that A. avenae multiplied rapidly on six of them. Hooper (1962) and Goodey and Hooper (1965) demonstrated the reproductive potential of A. avenae in agar cultures of mushroom mycelia. The former worker found that 20 A. avenae could destroy mushroom mycelium grown in petri dishes in three weeks while producing 60,000 progeny.

The latter workers demonstrated that a single larva of A. avenae was capable of reproducing to approximately 70,000 to 90,000 progeny on mushroom mycelia in three weeks time. Klink and Barker (1968) found that A. avenae destroyed the mycelium growing out of an initial sclerotium of Rhizoctonia solani. Cooke and Pramer (1968) demonstrated that A. avenae fed on five species of nematode-trapping fungi and noted that eventually A. avenae could, under certain conditions, kill the fungi. Monoson (1971) also found that A. avenae fed on four species of nematophagous fungi.

Mankau (1969) reported that in plates inoculated with both Rhizoctonia solani and Aspergillus niger, A. avenae survived only in areas occupied by R. solani. He also demonstrated that filtrates of A. niger caused immobilization of the nematodes. Evans and Fisher (1970b) reported that Rhizoctonia solani strain 48, grown on PDA or on Rhizoctonia medium, produced 711,400 A. avenae in 28 days at 25°C. A recent report of Evans (1970) showed that populations of A. avenae cultured on Rhizoctonia solani grown on cereals (oats, wheat, etc.) in wide-mouth preserving jars (680 ml) could reach 12,000,000 progeny per jar at 25°C in six weeks. Kondrollochis (1977) found that A. avenae reproduced readily on Botrytis cinerea and that it is less sensitive than Ditylenchus myceliophagus to the aging of its host.

From these workers' findings there is evidence that phytopathogenic fungi are better hosts for A. avenae than are saprophytic forms. The generation time for A. avenae under the most favorable

temperature conditions was about six days (Hechler, 1962; Evans and Fisher, 1970b) and the population reaches its peak in two weeks (Evans and Fisher, 1970a). Webster (1972) concluded that A. avenae from different isolates varies in size, internal morphology, fecundity and sex ratio, and that different hosts, temperatures and starvation stress may affect all these characteristics.

2. Damping-off of pea caused by Pythium species

A. The pathogens - Pythium species

Species of Pythium are world wide in their distribution and they occur in virtually all types of soil in which vascular plants are growing. The most common and important plant diseases caused by these fungi are pre- and postemergence damping-off of germinating seeds and emerging seedlings.

The taxonomic position of the genus and its relationships to other Phycomycetes were well established during the latter part of the nineteenth century. Middleton (1943) compiled an extensive monograph of the genus complete with host records and illustrations of the more important species. A comprehensive review of the Pythiums was made by Hendrix and Campbell (1973), and a more recent review of the taxonomic and genetic studies of them can be seen in Hendrix and Papa's (1974) publication. Perhaps the most complete literature list on species of Pythium and the diseases they cause was published by Tompkins (1975).

B. The diseases - damping-off of peas

Root rot and damping-off caused by species of Pythium, Aphanomyces, Ascochyta, Fusarium, and Rhizoctonia are the limiting factors in pea production in most pea growing areas. Pythium species, alone and in combination with other parasites, are considered to be the most important pathogens.

Early in 1937 Hull noted that reduction in a stand of pea seedlings was chiefly due to Fusarium and a Phycomycetous fungus, and that the emergence of pea seedlings was much reduced under conditions of high soil moisture, whereas soil temperature was a factor of less importance. He also found that wrinkle-seeded varieties were most susceptible to preemergence damping-off. Horsfall (1938) reported that in New York State the soil fungus principally involved in pre- and postemergence damping-off of peas was Pythium ultimum, Trow. He added that soil dampness was very important in disease occurrence. Padwick, in 1938, discussed "vigor" of seed samples and concluded that poor stands of peas were due to several pathogenic fungi. Hynes and Wilson (1939) attributed much of the poor emergence of peas in New South Wales to "low vigor." Baylis (1941) reported that fungal attacks on pea seedlings often took place at a very early stage in their development and that species of Pythium were obtained from almost every seedling axis. He attributed the problem principally to species of Pythium which he referred to as P. ultimum and P. debaryanum. Inoculation and field experiment results led Reinking (1942) to conclude that Pythium

ultimum could cause seed decay of pea as well as root rot in moist soil, together with stunting and death of plants.

Leach (1947) found that seed decay and preemergence infection of peas was severe at temperatures between 12 and 25°C, and that infection was most severe at temperatures that were relatively less favorable to the host than to the pathogen (Pythium ultimum). Hare (1949) demonstrated that Pythium ultimum in the soil killed 100% of pea seedlings in 10 days, after pre-treatment for 48 hours in the moist chamber. He also concluded that the optimum temperature range for pathogenicity was from 24 to 28°C. Angell (1950a, 1950b) reported that the fungus associated with pea blight in all soils in Australia is commonly referred to as Pythium ultimum Trow, which has more effect on yield of peas and poppies than does P. mamillatum. Middleton (1952) reported that 22 species of Pythium have been recovered from pea plants, and that P. apahnidermatum, P. irregulare, and P. ultimum cause seed decay. He was of the opinion that most seedling blights were produced by these species and that blights varied with variety of pea, the quantity and virulence of the fungi, the depth of seeding and soil temperature.

In 1953, Schroeder noted that various species of Pythium, notably P. ultimum, could parasitize the tissues of the underground part of peas to such extent as to kill it. He also found that the fungus rots the germinating seeds. He also noted that he could find no evidence of resistance when he tested various cultivars of pea with

P. ultimum. MacNeill (1956), in a study of pea root rot in Ontario, reported that Pythium ultimum was of major importance in the early part of the season with Fusarium solani taking over the major role later in the growing season. Saksena (1959) showed that South Australian soil carries a natural infestation of Pythium species, particularly P. debaryanum Hasse, which cause damping-off in peas. He also found that damping-off of wrinkle-seeded peas increases significantly with increase in soil moisture above 13.5%, and that at high moisture levels the seeds were most susceptible during the first 24 hours after planting. Pre-soaking the seeds in water for 12-14 hours reduced the diffusion from the seeds and thus increased emergence by 40%.

The "Index of Diseases in the United States," published by the U.S. Department of Agriculture (1960), listed 10 species of Pythium which are pathogenic to peas. The diseases they caused are listed as: root rot, damping-off, seed decay, pod rot and shoot blight. From the results of a survey made in Washington and Oregon, Hampton and Ford (1965) reported that an average of 80% of the pea plants observed in the previous year was moderately to severely root-rot infected. Isolation showed that species of Fusarium, Pythium, Aphanomyces and Rhizoctonia were involved in the disease. Harper (1966) found that Fusarium and Pythium species were the fungi most frequently isolated from diseased pea roots in southern Alberta, Canada. He mentioned that Pythium spp. were isolated from seeds germinated for 3 days at 15°C in moist, naturally infested soil. Escobar et al. (1967) reported that

Pythium spp. which were pathogenic to pea were obtained from 20 to 25 soil samples. Of the four isolates identified, two were P. ultimum and two were P. debaryanum. They also found all 42 commercial varieties tested by them were susceptible to these fungi. From a field survey, Kraft and Burke (1971) found that Pythium ultimum was the major fungus isolated from bean and pea fields in Washington. They also reported that the population of P. ultimum developed rapidly in land cropped to a susceptible host.

By testing 16 species of Pythium in a greenhouse trial, Robertson (1973) found that P. ultimum, P. debaryanum, P. irregulare, P. spinosum, and P. splendens were capable of causing damping-off of germinating seeds and seedlings of tomato, pea and morning glory. Burke and Kraft (1974) reported that P. ultimum, Rhizoctonia solani, and Thielaviopsis basicola had accumulated on beans and peas after 15 and 6 years on monoculture of the respective crops but that P. ultimum was the only prevalent pathogen which caused extensive necrosis.

From the findings by the above-mentioned workers, it can be summarized that the pre- and postemergence damping-off of pea seedlings is mainly incited by species of Pythium, and the incidence of the disease may be affected by such major factors as soil moisture, soil temperature, and the susceptibility of the cultivars. These and other concerned factors have been discussed and described in publications by Middleton (1943), Chupp and Sherf (1960), Zaumeyer (1962), Wheeler (1969), Walker (1969), Agrios (1969), and Garrett (1970).

There are few details on resistance in peas to attack by species of Pythium or other soil pathogens. Generally, it is agreed that wrinkle-seeded varieties are more prone to fungal attack than are the smooth-seeded varieties (Hull, 1937; Saksena, 1959; Flentje and Saksena, 1964; Short and Lacy, 1976, and others).

C. Control of damping-off of pea

The earliest method to improve seedling emergence of peas was a chemical seed treatment. Hull (1937) reported that by treating the dry seed with an organic mercurial compound, the stand of seedlings could be materially improved. Horsfall (1938) noted that the fundamental aspects of damping-off control were seed protection and soil treatment. He mentioned that water control and the ventilation of certain soils might help. By testing chemicals on peas for the control of soil fungi, Crosier (1946) found that the use of Arasan and Spergon reduced pea seed decay to only 5% in natural soil infested with Fusaria, P. ultimum and Rhizoctonia solani.

Schroeder (1953) reported that in infested pea growing soils, growers have to depend on seed treatment, crop rotations and good management of soil and crops. He further stated that seed treatment largely controls the seed decay and preemergence damping-off stages. Chupp and Sherf (1960) showed that the easiest and most effective control measure for any vegetable damping-off is to treat the seed and then to spray the seedlings and the soil at frequent intervals with a safe fungicide. Harper (1966) found that pea seeds treated with Captan,

Semesan and Bayer 47531 gave highest emergence and yield in southern Alberta. Walker (1969) noted that Captan is used widely as a protectant against the causal agent of damping-off and is especially recommended for seed treatment of peas. He emphasized that some plants are more susceptible than others to injury by a given material, therefore relative phytotoxicity is as important as fungicidal value. Recent findings of Robertson (1976) showed that seed treatment with carboxin, fenaminosulf, prothiocarb and Terrazole (echlomezol) enhanced seedling survival and yields of peas.

Fumigation with Chloropicrin or methyl bromide, or a combination of the two, is now standard practice in many nursery and horticultural operations. Gill (1970), Hendrix et al. (1970), Kraft et al. (1969), and Vaartaja (1967) reported that these chemicals do not kill all organisms. Species of Pythium and other pathogens multiply rapidly if re-introduced, and they offer greater threats to plants after than before fumigation. Hendrix et al. (1970) found that Dexon, and certain other soil fungicides, applied at low rates successfully retarded the rate of reinfestation of fumigated soils by Pythium.

A change in agricultural practices and environmental factors can sometimes have an effect on the incidence or severity of disease. Early in 1942, Reinking showed that crop rotation should be carried out after 3-5 years of planting the same soil with peas. Field soil should be properly prepared, sufficiently fertilized, well-drained and not previously planted to peas. Walker (1969) noted that rotation is of

little value in controlling the damping-off and root rot fungi because of the wide host range of the pathogens involved. Schroeder (1953), Zaumeyer (1962), and Agrios (1969) have also emphasized that soil with good external and internal drainage should be selected for peas. Monoculture should be avoided, and one should not return diseased pea straw into the soil.

Due to the wide host range of many species of Pythium, biological control and control of Pythium disease by resistant varieties are often not successful (Hendrix and Campbell, 1973; Wallace et al., 1975). However, a few resistant cultivars of peas with some resistance to Phycomycetes have been reported by Lockwood (1960), McDonald et al. (1961), Hagedorn (1973), Muehlbauer and Kraft (1973), and others.

3. The potential of Aphelenchus avenae for the control of soil-borne fungal diseases.

Aphelenchus avenae is a well known mycophagous nematode that is, in many cases, associated with root diseases. Although a few workers have found that this nematode has limited parasitic capability on higher plants and on plant tissues in vitro, its position as a parasite of higher plants has not been well determined.

A. Nematode-plant relationships

Christie and Arndt (1936) observed A. avenae to migrate beyond the diseased area of plant roots and into healthy cortical tissue. They indicated that this nematode may attack diseased plant tissues and utilize the contents of the cells in early stages of necrosis, but they

regarded it as being simply mycophagous or saprophytic. With histological evidence to support him, Steiner (1936) concluded that many populations of A. avenae live as saprophytes, but that they are also undoubtedly able to attack and damage healthy plant tissues (phlox hybrid) and to reproduce in tissue not yet decayed. Arndt and Christie (1937) concluded that the presence of the nematodes in the soil in large numbers may sometimes have a stunting effect on plants, and that the number and the severity of fungal-induced hypocotylar lesions increased in the presence of A. avenae. Goodey (1951) stated that A. avenae could penetrate and live in healthy plant tissues and he considered it to be a facultative parasite. Mello (1958) reported that A. avenae was found in healthy roots that had been stained. He therefore considered it to be parasitic in melon (Cucumis melo var. cantalupensis).

Thorne (1961) reported that A. avenae had frequently been found inhabiting the crown, leaf sheath, root cortex and other plant parts, where they appeared to be feeding on the contents of living cells. Decker (1962) found A. avenae in the roots of various plants, especially when grown in sandy soil, and it was more frequent in roots that were also infested with Pratylenchus spp. Barker (1963) reported that A. avenae was able to parasitize and reproduce on tobacco callus in vitro as well as on pot-grown Kentucky bluegrass. He found that the growth of the Kentucky bluegrass was greatly reduced by inoculating the five-inch pots of soil with 2,000 nematodes per pot. In the following year, Barker (1964) observed that there was no difference in the growth

of bean plants inoculated with as many as 100,000 A. avenae. He considered that this nematode was not parasitic to bean. Chin (1964) provided photographic evidence that A. avenae was capable of feeding on root hairs of corn, oats, beet, turnip, cabbage and radish. However, he could obtain no evidence of feeding on the root hairs of several other crop plants. Chin and Estey (1966) found that large populations of A. avenae caused stunting and wilting of cabbage and oats after 60 days of growth, but no evidence of nematodes could be found in the root tissues. Terry (1966) reported the presence of nematodes and eggs of A. avenae in roots of several cultivars of corn and tomato. He also observed a reduction of plant height in nematode-treated plants.

Barker and Darling (1965) found that A. avenae fed and reproduced readily on carrot, periwinkle, tobacco and tomato callus tissues and stated that these callus tissues were good hosts. Tikyani and Khera (1969) reported that A. avenae was reproducing well on lucerne callus as well as on several fungi. Klink (1966), Klink and Barker (1968) claimed that the root systems of beans and peas were freely invaded when large populations of A. avenae were present. They commented that the presence and reproduction of nematodes in the root system probably enhanced root deterioration. Porth (1975) found that A. avenae prevented nodulation on exised roots of Phaseolus vulgaris and that root damage by A. avenae was greatest when cells of Rhizobium phaseoli were present.

B. Nematode-plant root disease relationships

Many workers have claimed that A. avenae was frequently associated with other nematode or fungal diseases in nature. Norton (1959) found A. avenae present in the dry land where root rot trouble of small grains and native grasses had been reported. Chin and Estey (1966) reported that A. avenae was responsible for increase in severity of wilt induced by Verticillium albo-atrum in tomatoes. Terry (1966) observed that a combination of 40,000 A. avenae plus Verticillium dahliae gave a high disease percentage on sunflower seedlings. In "Index of Plant Diseases in the United States" of the U.S. Department of Agriculture (1960), A. avenae was recorded as having been associated with browning symptoms in narcissus but that it was probably a secondary invader. In the same report, A. avenae has also been recorded in roots of apples in Maine. Johnson and Boekhoven (1969) reported that A. avenae was infrequently isolated from greenhouse soils where tomato and cucumber were growing, and that it was considered to be a fungus-feeding species of no economic importance.

C. Aphelenchus avenae as a mycophagous nematode

Various workers have considered A. avenae to be a mycophagous nematode of the soil or rhizosphere environment and unable to reproduce on intact, healthy plant roots. Linford (1939) observed that three plant parasitic nematodes were attracted to fresh wounds of plant roots and pieces of green pineapple leaf, Portulaca oleracea stem, and tomato petiole, but A. avenae showed very little evidence of grouping responsiveness. Norton (1959) found that the build-up of A. avenae in

the soil was somewhat correlated with organic matter (cut wheat straw and roots) amendments. Chitwood and Berger (1960) noted that A. avenae was prevalent in the roots of coffee, but they thought it to be fungivorous and of no significance unless as a fungus vector.

Mankau and Mankau (1962) failed to detect any root damage of sweet orange seedlings by A. avenae despite additions of 10,000 to 200,000 larvae and adults. In another experiment with A. avenae, Mankau and Mankau (1963) could find no indication of bean root entry or damage by this nematode. They also stated that the occurrence of A. avenae in plant rhizospheres and in diseased tissue was probably due to the presence of fungal hyphae. Sutherland (1967) concluded that A. avenae was unable to penetrate and multiply on the roots of seven types of conifer seedlings. Sutherland and Fortin (1968) noted that A. avenae not only failed to enter roots of red pine, it was also incapable of destroying established mycorrhizae. They concluded that A. avenae is mainly a fungivorous nematode.

D. Influence of Aphelenchus avenae on root diseases

Because of the tendency of A. avenae to feed on fungal mycelia, its potential for the control of fungal-induced root diseases has been assessed by several workers.

The affinity of A. avenae for plant pathogenic fungi was indicated by Rhoades and Linford (1959), who found that 125,000 nematodes per pot of soil gave control of the Pythium root rot (Pythium

arrhenomanas) of corn. Barker (1964) studied the effect of A. avenae on reduction of disease due to Rhizoctonia solani on beans and showed that 100,000 nematodes per 5-inch crock gave almost complete control of this disease. Subsequently, Klink (1966) and Klink and Barker (1968) reported that about 4,000-6,000 A. avenae per 1 ml of fungal inoculum controlled root rots of pea and bean caused by Fusarium oxysporum f. sp. pisi and Rhizoctonia solani in pot trials. They also showed the ability of A. avenae to destroy inocula of certain other fungi in soil.

Roy (1973) showed that incidences of root disease of tomato caused by Rhizoctonia solani and Colletotricum coccodes was decreased by A. avenae in pot experiments with sterilized and unsterilized soils. He noted that best results were achieved with an inoculum of 26 nematodes per gram of soil and that inocula of both fungi were reduced in the presence of A. avenae. Cheng and Tu (1974) reported that experiments with various fungus-nematode combinations showed that initial populations of 1,710 to 8,550 of A. avenae gave the best control of flax and jute damping-off in 133 cu cm soil infested with Rhizoctonia solani. Porth (1975) found that populations from 3,300 to 13,200 individuals of A. avenae controlled preemergence damping-off of beans incited by Sclerotinia sclerotiorum. He also noted that no effect on plant growth by the nematode was observed.

III. ISOLATION OF PYTHIUM SPECIES FROM LOCAL SOIL

Species of Pythium cause pre- and postemergence damping-off of many vegetable seedlings. An attempt was made to isolate the species from local soil with the object of selecting the most virulent one for use throughout this study. In September, 1976, the first soil samples were collected and the isolation process for Pythiums was started.

A. Materials and methods

Four soil samples were taken, with the aid of a trowel, from the root zone of bush bean, zucchini, potato and cauliflower from Plant Pathology field plots at Macdonald College. The four samples from root zones of the same cultivar were mixed together thoroughly and then placed into a 28 cm x 36 cm x 8 cm plastic tray which was kept separate from each of the other composite soil samples.

Two baiting methods were used for isolating Pythium from the soil. The first method involved the direct seeding of susceptible plant seeds in the root-zone soils.

Seeds of tomato, cucumber, bean, pea and radish were surface sterilized in 1:9 Javex (6% NaOCl)-water solution for 3-5 minutes, rinsed once and soaked in sterile distilled water. After 24 hours of soaking, seeds of each vegetable were planted in one row per tray of soil. To compare this method with a normal greenhouse seeding process, one tray of natural soil from the greenhouse stock was used and planted

with treated cucumber and pea seeds only. All trays were watered heavily after seeding and they were left on a bench in the greenhouse where the temperature ranged from 27 to 30°C.

The preemergence damped-off seeds and portions of the tissues from postemergence damped-off seedlings or infected roots were sampled. These were washed in running water for a few minutes, excess surface water was absorbed with filter paper, then they were plated on a Water Agar Medium (WA) containing 1% agar, and incubated at 25-28°C in an incubator until fungi grew from the plant material.

The second method was one described and used by Goth et al. (1967) for baiting sugar fungi from the soil by using boiled plant seeds. Sweet corn seeds were soaked for 24 hours in distilled water autoclaved at 121°C for 30 minutes. After cooling, five seeds were sown, 2 cm deep, in a 10-cm pot containing soil from the various trays. The pots were watered heavily after seeding and a polyethylene sheet was applied on the top of these pots to retain the moisture. The seeds were dug out after two days, washed, wiped dry and one seed per dish was plated on WA and incubated at 25-28°C.

Pure Pythium cultures were obtained by transferring the hyphal tips of these fungi as soon as they emerged from the plated materials. Fungi other than Pythium were also isolated. A technique outlined by Sleeth (1945) was used to eliminate bacteria and other undesirable contaminants, when this was necessary.

Sleeth's medium contained:

Dextrose	10 g
Ammonium acid phosphate	2 g
Potassium	1 g
Magnesium sulphate	1 g
Agar	25 g
Water, distilled	1,000 cc

Purified cultures of Pythium and other fungi were maintained on 2/3 strength Difco Potato Dextrose Agar (PDA). Every isolate was assigned a number and a record was made of its source. To avoid loss of pathogenicity, 3-4 surface-sterilized seeds of corn or pea were added to these stock cultures of Pythium after several subcultures had been made.

B. Results

From the above two isolating methods, twelve pure cultures of Pythium (Table 1) and other fungi, such as Fusarium (3 isolates) and Rhizoctonia (5 isolates) were obtained.

There were few damped-off seedlings except with the susceptible cucumber and peas. Preemergence killing occurred more frequently on bean than on the other seeds.

The sweet corn baiting method was found to be very useful for isolating fast-growing fungi, like Pythium species, from the soil, and Sleeth's medium was very effective in eliminating contaminants.

Table 1. Cultures of Pythium isolated from local soil.

Isolate number	Origin	Detected by or isolated from
P1	Zucchini	Corn kernel
P2	Cauliflower	Cucumber seedling
P3	Zucchini	Corn kernel
P4	Potato	Bean seed
P5*	Carolina Biol.Supply Co.	------
P6	Zucchini	Cucumber seedling
P7	Greenhouse soil	Pea seedling
P8	Potato	Cucumber seedling
P9	Greenhouse soil	Cucumber seedling
P10	Zucchini	Corn kernel
P11	Zucchini	Corn kernel
P12	Zucchini	Bean seed
P13	Zucchini	Bean seed

* This named culture was included for comparative purposes.

IV. PATHOGENICITY TESTS OF PYTHIUM ISOLATES

To select the most pathogenic isolate of Pythium, a preliminary study was made of their growth characteristics. They were also compared with a culture of Pythium debaryanum obtained from the Carolina Biological Supply Co. (no. 156214), and with each other in a simple laboratory test of pathogenicity, in petri dishes. Only those which showed strong pathogenicity in the petri dish tests were selected for the greenhouse tests.

A. Materials and methods

The isolates of Pythium were grown on 2/3 strength PDA at 28°C for five days before studying their growth characteristics, and their spore-producing capability. The production of spores on the 2/3 strength PDA was compared with cultures grown on carrot agar (Tuite, 1969).

The laboratory test of pathogenicity was done by plating five surface-sterilized, 24-hour-soaked (as on page 18) pea, cucumber or radish seeds on the mycelium of each of the fungi grown on PDA. After five days of incubation at 25°C, the number of dead and germinating seeds was recorded.

After the isolates had been screened in the petri dish test, eight of the most virulent ones were selected for a postemergence

damping-off test. This was done by first surface-sterilizing water-soaked seeds (as on page 18) of cucumber (var. Market More 70) and pea (var. Little Marvel) and then planting three of each variety per 10-cm clay pot of steam-sterilized soil (one part sand, two parts soil, pH 6.5). The resulting seedlings were allowed to grow for 12 days in the greenhouse, when an inoculum of the fungus was introduced into the soil of each pot.

The inoculum was prepared from cultures of the Pythium isolates grown on PDA at 28°C for 8-10 days. A mycelial suspension was obtained by macerating the mycelium scraped from each culture dish, with 100 ml of distilled water for 5-7 seconds in a Waring Blendor. Fifty ml of the mycelial suspension was poured into the soil of each pot and the surface of the pots was covered with a layer of sterilized fine soil. The checks received only plain PDA instead of inoculum.

Inoculated pots were arranged randomly on a perforated metal shelf suspended inside a water tank measuring about 235 cm x 73 cm x 60 cm (Figure 1). The tank was filled with a layer of water (8-10 cm depth) at the bottom and a polyethylene sheet was applied on top to provide sufficient moisture for disease development. The temperature inside the tank ranged from 24 to 27°C and the relative humidity was between 70 and 80%, which was measured by a hygrothermograph. Artificial illumination was supplied to the plants at about 13,000-14,000 lux for 16 hours each day. A record was kept of the number of damped-off and non-damped-off seedlings during the experimental period,

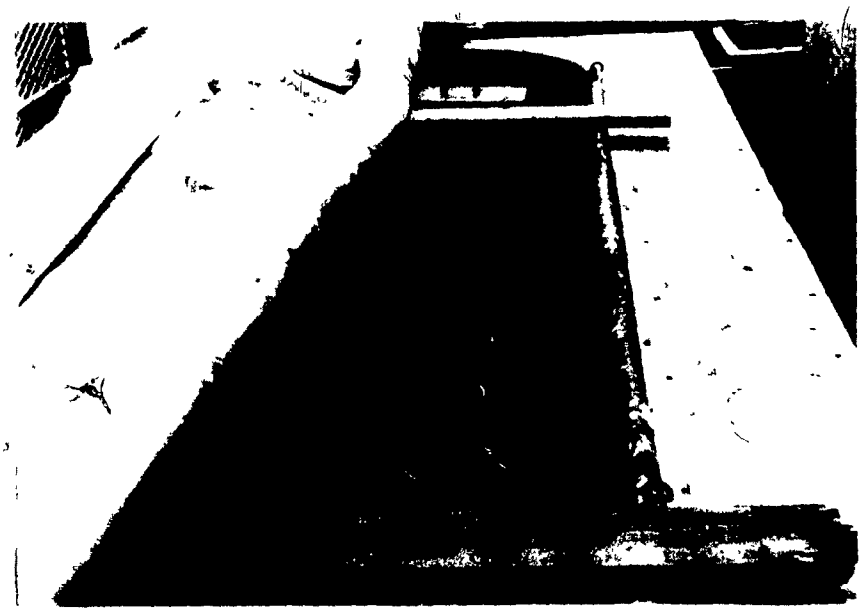

Figure 1. The water tank used for the damping-off experiments.

which was terminated 12 days after inoculation. This, and some preliminary trials, showed that little or no change in the results occurred after about the tenth day. For this reason, all damping-off experiments were terminated on the twelfth day. To be certain of the cause of damping-off, the fungus was reisolated from the affected seedlings.

The experiment consisted of eight treatments and each treatment was replicated four times, and the entire experiment was repeated twice.

B. Results

From the above-mentioned isolation experiments, fungi with loose cottony-type mycelium were in the majority (Table 2, Figure 2). Most of the isolates sporulated well on 2/3 strength PDA although a few of them did not. Production of conidia, oogonia and oospores was more abundant on the carrot agar medium than on PDA. Allantoid bodies (Sideris, 1932) or frustrated appressoria (Tompkins et al., 1939) on the hyphae were observed abundantly in some isolates.

In petri dish tests, most isolates showed strong pathogenicity on germinating seeds of cucumber and pea (Table 2) but they were only weakly pathogenic to seedlings of radish.

In greenhouse tests, all the isolates were pathogenic to more than 50% of the pea seedlings, whereas only two isolates were pathogenic to cucumber in experiment 1. Results obtained from experiment 2 showed that all isolates were uniformly pathogenic to both pea and

cucumber. Many seedlings were damped-off just two days after the inoculation (Figures 3 and 4).

On the whole, it was observed that seedlings of pea and cucumber were very susceptible to the Pythium isolates tested. However, a fast-growing crop like cucumber becomes resistant to fungal attack earlier than does that of the slower growing pea, since plant seedlings are extremely susceptible to Pythium at the very early stage (Chupp and Sherf, 1960; Wheeler, 1969; Walker, 1969; Garrett, 1970).

From the above results, it was learned that the damping-off of seedlings caused by Pythium had been well postulated and that it was possible to reproduce the results under similar conditions in the greenhouse, using the same dosage, cultivar and seedling age. The experimental methods were standardized for the succeeding experiments.

Because several workers (Baylis, 1941; Flentje, 1964; Flentje and Saksena, 1964; Escobar et al., 1967; Short and Lacy, 1976, and others) have indicated that preemergence damping-off is related to sugar content or exudations, a simple test for sugars was carried out, as described by Fieser (1968).

The results of this test showed that young stems of pea seedlings contain as much or more sugar than do roots of a comparable age.

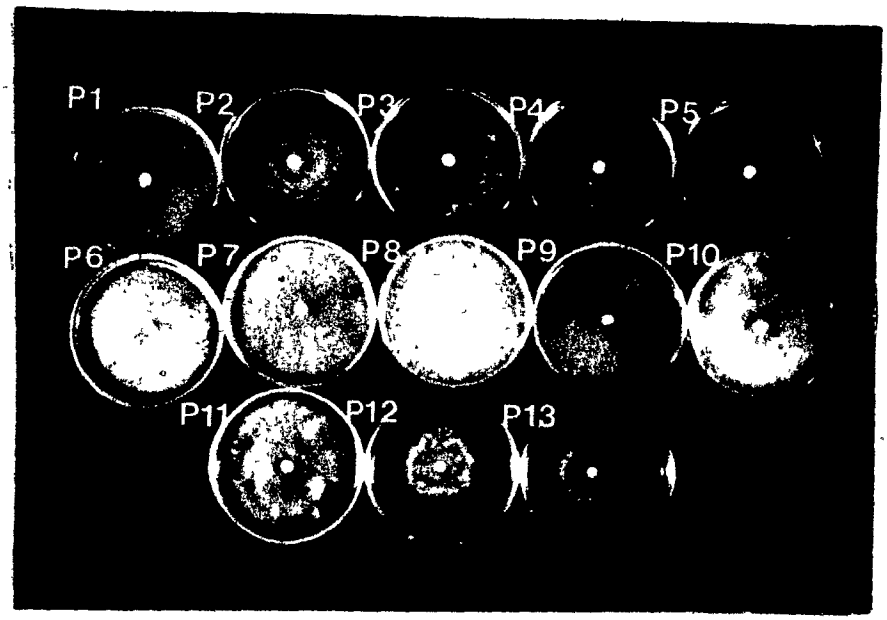

Figure 2. Characteristics of Pythium isolates, grown on 2/3 PDA.

Figure 3. Seedlings of cucumber (A) and pea (B) infected by Pythium isolate P3, two days after inoculation.

Figure 4. Shrunk and water-soaked symptoms (arrows) on stems of damped-off pea and cucumber seedlings two days after being inoculated with Pythium isolate P3. Plants on the extreme right and left are not infected.

Table 2. Characteristics of the Pythium isolates grown on 2/3 strength Difco Potato Dextrose Agar.

Isolate No.	Growth * type	Sporulation	Pathogenicity on:**		
			Pea	Radish	Cucumber
P1	Radial	Yes	+++	-	+++
P2	Rosette	No	+++	-	+++
P3	Cottony	Yes	+++	++	+++
P4	Cottony	Yes	++	++	+++
P5	Cottony	Yes	++	++	+++
P6	Cottony	Yes	+++	++	+++
P7	Radial	No	++	++	+++
P8	Cottony	No	+++	++	+++
P9	Radial	Yes	++	++	++
P10	Cottony	No	++	-	+++
P11	Cottony	Yes	++	-	+++
P12	Rosette	Yes	-	-	++
P13	Rosette	Yes	-	-	++

* These are illustrated in Figure 2.

** Five seeds were plated on mycelium of the fungus grown in petri dish. Ratings were made 5 days after plating.

Rating methods : +++ Three or more seeds killed.
++ One or two seeds killed.
- No seeds killed.

Table 3. Occurrence of damping-off on pea and cucumber inoculated with *Pythium* isolates.

Isolate *	% of seedlings damped-off			
	Pea		Cucumber	
	Expt. 1	Expt. 2	Expt. 1	Expt.2
P1	66	58	0	58
P2	50	41	0	66
P3	100	100	83	75
P4	66	83	0	41
P5	50	66	0	25
P6	50	58	0	58
P7	50	75	0	50
P8	100	83	8	50

* These isolates were selected from previous in vitro tests.
** Four replicated treatments of 3 plants/treatment. Data were collected 12 days after inoculation.

V. STUDIES ON THE MULTIPLICATION OF APHELENCHUS AVENAE ON SIX ISOLATES OF PYTHIUM

Mankau and Mankau (1969) demonstrated that neither Pythium species nor Phytophthora species were good hosts for A. avenae. Rhoades and Linford (1959) and Chin (1964) found that A. avenae could feed and multiply on P. arrhenomanas and P. debaryanum, respectively. This study was carried out to determine the reproductive capability of A. avenae on selected isolates of Pythium.

A. Materials and methods

Six isolates of Pythium which showed high pathogenicity to pea in the previous tests were selected for a study of their suitability as hosts for A. avenae. One species of Fusarium and one of Rhizoctonia were included in the test series for comparative purposes.

The fungal hosts were cultured on 2/3 strength PDA in glass petri dishes. Each dish was inoculated with 50 nematodes when the mycelium of each fungus had almost covered the surface of the medium. Because the growth rate of the species of Fusarium was much slower than that of the other fungi, it was cultured a week earlier than others.

The A. avenae, obtained from a stock culture in the laboratory, was maintained on Rhizoctonia solani on 1/2 strength PDA. The nematodes were extracted from the fungal mycelia by use of a modified

Baermann extraction pan (Townshend, 1963). They were washed through a 25-µm sieve with distilled water, to remove fungal and nematode metabolites, and then surface-sterilized by the method described by Hooper (1970). They were first washed in the surface-sterilizing solution (Streptomycin sulfate, 0.1% plus 20 ppm Malachite green in a 10-ml Syracuse watch glass) for two hours, after which they were rinsed four times with sterile distilled water. The desired number of nematodes was added to the fungal culture in a drop of sterile distilled water. The culture dishes were then incubated at 25°C in an incubator with no illumination supplied.

Later, the nematodes were recovered from the culture dishes and their number was estimated. This was done by counting the nematodes in a 10-ml suspension of them in a 5.5-cm plastic dish with indicating lines drawn on the bottom. The counting of the nematodes was performed under a stereoscopic microscope. The nematodes in one-fourth of the area were counted and the total number of nematodes was calculated after simple multiplication, with reference to the total volume. Each experiment consisted of six treatments. The treatments in experiment 1 were replicated five and those of experiment 2 six times. Experiment 1 was terminated 40 days after inoculation and experiment 2 was terminated in 20 days.

B. Results and discussion

The results of the above experiments were summarized in Table 4. The population of A. avenae on all Pythium isolates was lower in the 40-day culture period than in the 20-day period. However, it was found that A. avenae was able to reproduce on all isolates of Pythium in the test series, although the number recovered from most cultures was not as high as that from either the Fusarium or the Rhizoctonia isolates. Among the Pythium isolates, P3 was found to support a slightly higher population of A. avenae than any of the others in both experiment 1 and 2.

The above results supported the contention that A. avenae is able to multiply on Pythium, even though Mankau and Mankau (1969) reported a negative result. This apparent contradiction may be due to the use of different strains of Pythium or of A. avenae under a different set of environment conditions.

Table 4. Multiplication of *Aphelenchus avenae* on selected *Pythium* isolates cultured on 2/3 strength Potato Dextrose Agar.

Fungi	Initial No. of *A. avenae*	Final No. of *A. avenae*	
		Expt. 1*	Expt. 2**
P2	50	2,904	4,326
P3	50	4,072	4,611
P5	50	1,016	3,833
P6	50	2,176	4,386
P7	50	1,224	2,021
P8	50	1,190	4,365
Fusarium sp.	50	3,552	6,187
Rhizoctonia sp.	50	1,550	3,226

* Average of 5 dishes, period 40 days.
** Average of 6 dishes, period 20 days.

VI. STUDIES ON *PYTHIUM ULTIMUM* AND ITS RELATIONS WITH *APHELENCHUS AVENAE*

From the previous experiments, *Pythium* isolate P3 was found to be the most pathogenic of all the isolates tested against pea; therefore it was selected as the test fungus, and a wrinkle-seeded pea (var. Little Marvel) was used as the test plant throughout the remainder of this study.

1. Identification of the isolate P3

A. Materials and methods

Observations on the growth characteristics of *Pythium* isolate P3 were made by growing this fungus on 2/3 strength PDA at 28°C. The characteristics of its mycelium were observed. Carrot agar medium (Tuite, 1969) was used to stimulate sporulation of the fungus, as an aid to its identity. Observations were made after the fungus had been incubated for 3-5 days. The glass petri dishes were inverted on the stage of a light microscope and observations were made directly through the bottom of the glass dish. Germination of the resting spores was observed by first wetting the old cultures (2 weeks or older) with sterile distilled water and incubating them at room temperature (25°C) for a few hours.

Identification of the fungus was made with the help of the keys published by Middleton (1943), Waterhouse (1968) and Takahashi (1970a, 1970b) after tracing its morphological features.

B. Results and discussion

The isolate P3 produced a loose cottony, snow-white mycelium of aerial hyphae. It produced an abundance of terminal and intercalary conidia, oogonia and oospores, with monoclinous antheridia adjacent to the oogonia (Figure 5A). The oogonia were smooth, and when fertilized they became thick-walled oospores, as shown in Figure 6A. The fungus produced many allantoid bodies or frustrated appressoria in the culture (Figure 5B). The resting spores of the fungus were observed to germinate within two hours at room temperature (Figure 6B) but no formation of zoospores was observed. Based on these characteristics plus its morphological features, isolate P3 was identified as Pythium ultimum Trow.

According to many reports, including those of Middleton (1943, 1952), Angell (1950a, 1950b), Escobar et al. (1969), Kraft and Burke (1971), and Burke and Kraft (1973), P. ultimum is a very common species in North America and therefore could be the prevalent Pythium species on many plant seedlings. Because the spores of P. ultimum are capable of germinating in a very short time, requiring only a suitable moisture level at a favorable temperature, it is believed that this species plays an important role in disease development in germinating seeds and growing seedlings of various plants.

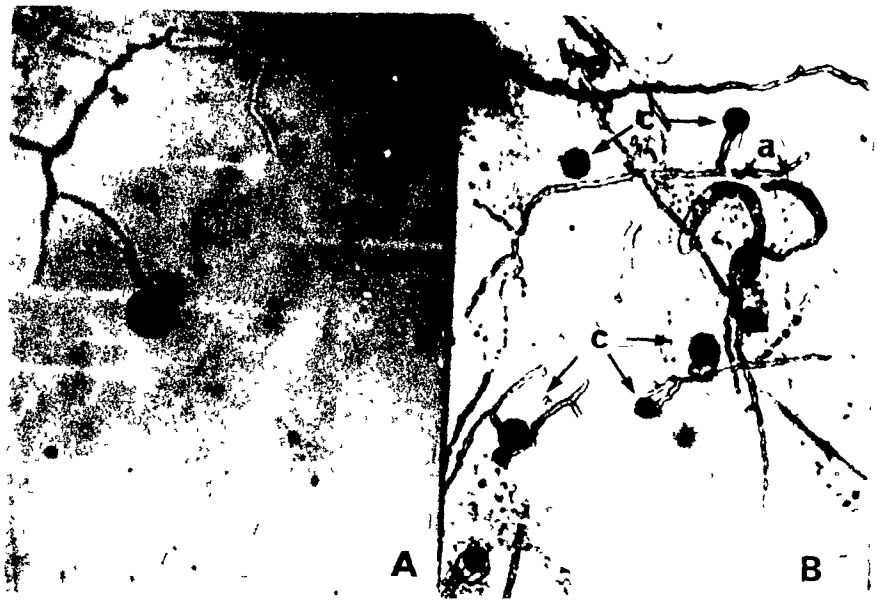

Figure 5. (A) An oogonium of Pythium isolate P3 with a monoclinous antheridium. (B) a : allantoid bodies or frustrated appressoria, c : conidia.

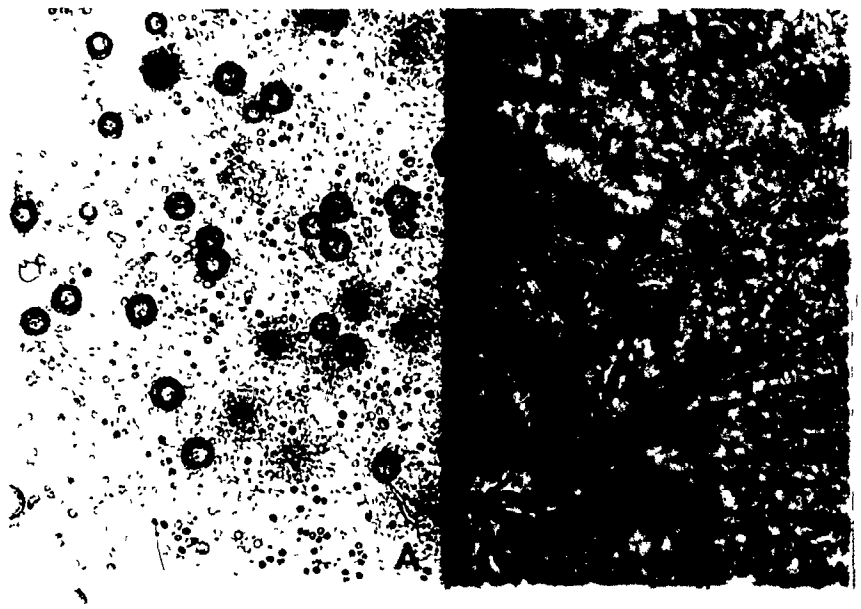

Figure.6. (A) Resting spores of Pythium isolate P3. (B) Germinated resting spore with at least three hyphae (arrows).

2. Observations on the feeding of Aphelenchus avenae on Pythium ultimum and two other fungi

A. Materials and methods

To observe the feeding behavior of A. avenae on P. ultimum, one nematode was placed on mycelium of the fungus grown on a thin layer of water agar in a cavity slide. A glass coverslip was applied over the surface of the cavity slide to prevent drying of the medium during the observation under a light microscope. Two species of other fungi, namely one of Fusarium and one of Rhizoctonia were also used for the purpose of comparison.

A stop watch, indicating 1/10 seconds, was used to record the feeding time of A. avenae. Feeding was assumed to have begun when the valve of the median bulb of the nematode started to pulsate, and to terminate when the pulsation of the valve ceased. At least 50 observations were made of the feeding time on each fungus.

B. Results and discussion

A. avenae started to feed on P. ultimum, Fusarium and Rhizoctonia almost as soon as it was released onto the hyphal mass. The feeding behavior was the same as that which had been observed and described by Chin (1964), and Fisher and Evans (1967).

A. avenae was seen to feed on every part of the hyphae. It moved freely in the mycelial mass and when it came in contact with one of the hyphae, it usually brushed its lips on the hyphal wall approximately at right angles to the axis of the hypha just prior to

thrusting its stylet and puncturing the hyphal wall. When it started to feed, the fungal protoplasm could be seen to flow into its esophagous through its stylet (Figures 7A, 7B, 8A, 8B).

A. avenae was observed to feed on allantoid bodies or frustrated appressoria of P. ultimum, as shown in Figure 8A, but not on oospores, because it could not puncture the thick walls of these spores. A. avenae spent more time feeding on P. ultimum than on either of the other two fungi (Table 5).

A. avenae commonly removed so much protoplasm from the hypha of P. ultimum that it would collapse either during or after feeding. When the nematode withdrew its stylet it was not unusual to see fungal protoplasm gushing from the wounds on both hyphae and allantoid bodies (Figures 9A, 9B). This feeding probably causes serious damage to colony development, as Rhoades and Linford (1959) had found before. The feeding on allantoid bodies may have an effect on infectivity of P. ultimum. A. avenae usually fed at one feeding site for quite a long time because there is no septum (coenocytic) in young hyphae of P. ultimum, protoplasm flows from both directions to the stylet, so it could keep on sipping at one site without moving to other cells.

When feeding on Fusarium or Rhizoctonia the nematode had to move from cell to cell after it had extracted the protoplasm from between septa (Figures 7A, 7B). Moreover, the damage caused by the feeding of A. avenae can be readily seen in those cells on which it had been feeding, in contrast to the undamaged neighbouring cells (Figure 7).

Table 5. Duration of feeding time of *Aphelenchus avenae* per feeding site on three fungi.

Fungi	No. of observations	Average time
Fusarium sp.	52	2.2 Sec.
Rhizoctonia sp.	52	3.9 "
Pythium ultimum	60	6.5 "

Note : Observations were made of the nematode feeding on mycelium of each fungus grown on a thin layer of water agar in a cavity slide.

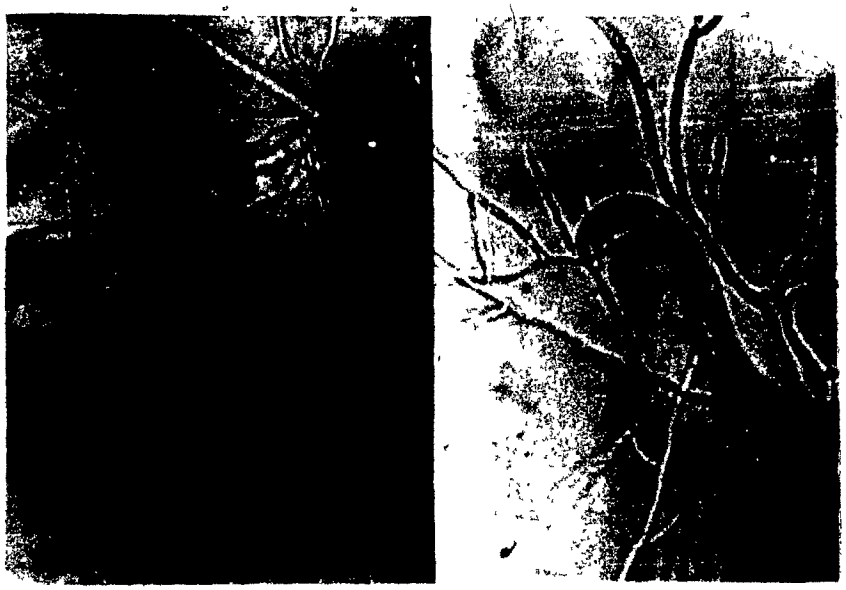

Figure 7. Aphelenchus avenae feeding on a hypha of Fusarium (A) and Rhizoctonia (B). Arrows indicate emptied cells.

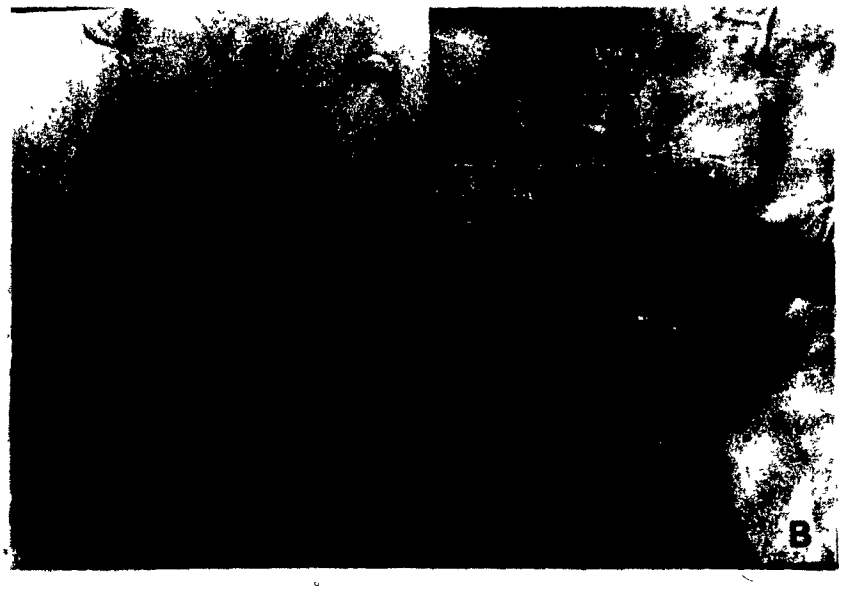

Figure 8. Aphelenchus avenae feeding on an allantoid body or frustrated appressorium (A)and on a hypha of Pythium ultimum (B).

Figure 9. A hypha (A) and an allantoid body or frustrated appressorium (B) of Pythium ultimum discharging protoplasm (arrows) from wounds made by Aphelenchus avenae.

VII. STUDIES OF POTENTIAL PARASITISM OF *APHELENCHUS AVENAE* ON PEA SEEDLINGS

Because *A. avenae* has been reported to have the ability to penetrate and to feed on higher plants, it was necessary to demonstrate that it would not parasitize pea seedlings, otherwise it would be of little value in the control of a pea seedling disease. The studies were carried out in axenic culture and in soil-grown pea seedlings.

1. Response of pea seedlings to *A. avenae* in culture

A. Materials and methods

Pea seeds (var. Little Marvel) were surface-sterilized and soaked in sterile water in petri dishes as previously described. The seeds were transferred onto 2/3 strength PDA until the radicle of the seeds had grown out about 1 cm. Seeds from which fungi or bacteria grew were eliminated immediately. Clean seeds, with radicles uniformly about 1 cm long, were transferred onto "soil extract agar medium," one per 100 mm x 20 mm glass petri dish. The seed coats were removed before transferring the seeds onto this new medium.

The soil extract agar medium was prepared according to Tuite's formula which contains:

Agar	7.5 g
KH_2PO_4	0.2 g
Soil extract	100.0 cc
H_2O	900.0 cc
pH - 6.8-7.0	

The soil extract stock solution was prepared by autoclaving equal volumes of field soil and water in a flask at 121°C for 30 minutes. When cool, the debris was removed and the liquid was filtrated. This fluid constituted the soil extract.

When planting, the radicle of the seed was manually inserted into the medium so that the roots of the seedling could grow down to the bottom of the dish. The dishes were incubated at 25°C in the dark.

Nematode inocula were prepared by the method previously described on pages 31 and 32. The desired number of nematodes, i.e., 10, 50, or 100 individuals per dish, were added when the seedlings were about seven days old. The seedlings were then incubated at 25°C in the dark. To avoid contaminating the test culture, extra dishes with the same number of nematodes were used for observation of possible feeding by A. avenae on these seedlings. Observations were made under a light microscope.

The experiments consisted of three treatments, each treatment being replicated 5 times in experiment one, and 6 times in experiment 2. Both experiments were terminated 20 days after inoculation.

After termination of the experiments, the nematodes were extracted by Baermann funnel and the final number of nematodes was determined by the same method as previously described on page 32. The roots of all seedlings were harvested and stained by the methods described by Hooper (1970). The roots were plunged into boiling lactophenol-cotton blue for 2-3 minutes. After cooling, they were

destained by washing with water and by placing them in clear lactophenol. The root tissues were examined for the presence of A. avenae by pressing the roots between two thin glass plates and examining them under a stereoscopic microscope.

B. Results and discussion

The axenic cultured pea seedlings grew well in the petri dishes containing soil extract agar medium, as shown in Figure 10. The numbers of nematodes recovered from the cultures 20 days after inoculation are shown in Table 6. Although nematodes were seen in close proximity to the seedling roots and the area of the root cap (Figure 11), they were not seen to penetrate or to feed on the root cells of the pea seedlings. By examining the stained root specimens it was confirmed that no nematodes had penetrated the root tissues.

The population of A. avenae was found to decrease greatly in axenic cultured pea seedlings. Most of the nematodes extracted from the cultures appeared to be very weak. They moved slowly and had an abnormal number of internal bubbles or vacuoles. It was felt that because A. avenae did not feed on the pea seedlings, they had to utilize their own internal energy sources, and in doing so, they had become virtually exhausted. The present results do not agree with the findings of Barker and Darling (1965) or of Chin and Estey (1966), who reported that A. avenae fed on plant tissues, excised roots and root hairs of many plants. It may well be explained that strains of A. avenae react differently in the presence of plant roots or that some strains feed on roots and some do not.

Figure 10. Pea seedlings growing in petri dishes on soil extract agar medium to which nematodes had been inoculated.

Figure 11. Root tip of a pea seedling with probing Aphelenchus avenae (arrow).

Table 6. Number of Aphelenchus avenae recovered from axenic cultures of pea seedlings, after 20 days.

Original population	No. of A. avenae recovered	
	Expt. 1 *	Expt. 2 **
10 A. avenae	14	7
50 A. avenae	16	10
100 A. avenae	26	23

* Average from five 100 mm x 20 mm glass petri dishes.

** Average from six " " " " "

2. Response of pea seedlings to Aphelenchus avenae in soil

This experiment was carried out to determine the response of pea plants to three population levels of A. avenae in soil under greenhouse conditions. The aim was to obtain further evidence to support the results obtained in previous experiments in culture dishes.

A. Materials and methods

Pea seeds were surface sterilized by the same method as previously described on page 18. One seed per 15-cm clay pot was sown in steam-sterilized soil (two parts sand, one part soil, plus 3 g 10-10-10 fertilizer per pot). The clay pots were put into larger plastic pots which helped to retain the moisture of the soil (Figures 14 and 15). The resulting seedlings were grown under normal environmental conditions in a greenhouse.

Nematodes were extracted from fungal mycelia by a modified Baermann extraction pan (Townshend, 1963). They were collected after 24 hours and washed through a 25-µm sieve with distilled water to remove the fungal and nematode metabolites. They were then collected into a beaker where the number of nematodes in the water was estimated by the method described on page 32.

Inoculations were made when the seedlings were about 2 weeks old (3-5 cm in height). The desired number of nematodes was added to each pot by pouring the nematode suspension into a small hole made with a glass rod very close to the base of the plant. The nematode suspension

was stirred by a glass rod for a few seconds to make the suspension homogeneous before taking any from the beaker. The control pots received only water instead of the nematode suspension.

After inoculation, the pots were arranged in a randomized block on a bench in the greenhouse. They were watered regularly with tap water. A wire was inserted into the soil of each pot to support the plants. All visible external abnormalities were recorded during the growing period. The plants received mainly the natural light from outside, but 13,000-14,000 lux of artificial illumination was supplied whenever it seemed to be necessary.

The experiments consisted of four treatments, each treatment was replicated six times for experiment 1 and five times for experiment 2. In experiment 1, plants were treated with 5,000, 10,000 and 50,000 nematodes and those of experiment 2 were treated with 25,000, 50,000 and 75,000 nematodes per 15-cm pot.

The experiments were terminated 30 days after inoculation. Plant heights were measured, then they were harvested and uprooted. The roots were washed in running water until they were free of soil and debris. The above ground part of the plants and their roots were weighed while still moist, then a 3-g sample was randomly removed from each root. These root samples were stained in lactophenol-cotton blue, as described on pages 44-45, and examined for the presence of nematodes.

The above-ground parts and the plant roots were separated and dried in an aerated oven at 80°C for 24 hours. Their dry weights were

determined and recorded after they had cooled for 2 hours. The dry weight of the 3 g of root which had been sampled before drying was calculated and added to the weight of the root from which it had been taken.

A 100-g sample of the soil in each pot was taken and after extracting the nematodes by Baermann funnel, the total number of nematodes per pot was estimated. Analysis of variance by F-test and Duncan's multiple range test was applied to the data for finding the significance between the treated and untreated plants.

B. Results and discussion

In experiment 1, the plant growth appeared to have been slightly reduced by the increased numbers of nematodes used, but the analysis showed that there was no significant difference between control and nematode-treated plants, either in plant height, the fresh or dry weight of the above-ground parts or the plant roots (Table 7). Table 8 shows the number of A. avenae recovered from the treated pots 30 days after inoculation. It is obvious that the number of nematodes recovered from the soil was very low in all cases, and that little, if any, multiplication had occurred. There were no nematodes to be seen inside the root specimens.

In experiment 2 there was no significant difference between control and two other treatments in which lower numbers of nematodes were added. But there was a negative significant difference between

the three treatments (including control) and the treatment in which the highest number of nematodes was added (Table 9).

The number of nematodes recovered from the nematode-treated soil followed the same tendency as in experiment 1. Although more nematodes were added in these treatments, in each case the number of A. avenae recovered was much lower than the number added. Again no nematodes could be found in the roots of the pea seedlings (Table 10).

A graphic comparison of differences in plant growth between experiments 1 and 2 is shown by Figures 12 and 13. It is known that pea is a cool temperature crop which grows well in the early spring. Experiment 1 was carried out in early spring, whereas experiment 2 was carried out in the summer, therefore it was not surprising to find that the plants in experiment 1 grew better (Figure 14) than those in experiment 2 (Figure 15). The former plants had a bigger root system (Figure 16) than the latter (Figure 17).

As is shown in Figure 17, although the plants treated with the highest number of nematodes produced larger and taller above-ground parts, their root systems were not much larger. It is difficult to explain how the plant growth was stimulated, but it apparently resulted from some direct or indirect effect of the presence of A. avenae.

Table 7. Pea plants exposed to three levels of *Aphelenchus avenae* in the soil, 30 days after inoculation. Expt. 1.

Treatment	Effects on plant growth *				
	Plant ht. (cm)	Plant top wt. (g)		Plant root wt. (g)	
		Fresh	Dry	Fresh	Dry
Control	20.05 a	17.86 b	2.99 c	10.73 d	0.80 e
5 x 10^3 *A. avenae*	18.37 a	16.28 b	2.86 c	10.25 d	0.70 e
10 x 10^3 *A. avenae*	17.52 a	14.82 b	2.62 c	8.87 d	0.70 e
50 x 10^3 *A. avenae*	16.48 a	18.40 b	3.06 c	10.47 d	0.71 e

* Average of 6 replicates. Means under a common letter are not significantly different by Duncan's multiple range test. (P = 0.05)

Table 8. Average number of Aphelenchus avenae recovered from the soils and plant roots, 30 days after inoculation. Expt. 1.

Treatment	No.* of A. avenae/pot	No.** of A. avenae/ 3 g root
Control	-	-
5×10^3 A. avenae	458	0
10×10^3 A. avenae	906	0
50×10^3 A. avenae	6,944	0

* Average of 6 replicates. Each pot contained approximately 800 g of soil. The number of A. avenae per pot was estimated from 100 g soil samples.

** Root samples were stained in lactophenol-cotton blue and examined under stereoscopic microscope.

Table 9. Pea plants exposed to three levels of *Aphelenchus avenae* in the soil, 30 days after inoculation. Expt. 2.

Treatment	Effects on plant growth *				
	Plant ht. (cm)	Plant top wt. (g)		Plant root wt. (g)	
		Fresh	Dry	Fresh	Dry
Control	13.58 a	4.95 c	0.75 e	3.15 g	0.15 i
25 x 10^3 *A. avenae*	13.18 a	6.20 c	0.85 e	2.32 g	0.12 i
50 x 10^3 *A. avenae*	14.70 a	6.21 c	0.93 e	2.55 g	0.14 i
75 x 10^3 *A. avenae*	23.10 b	9.08 d	1.24 f	5.33 h	0.26 j

* Average of 5 replicates. Means under a common letter are not significantly different by Duncan's multiple range test. ($P \leq 0.05$)

Table 10. Average number of Aphelenchus avenae recovered from the soils and plant roots, 30 days after inoculation. Expt. 2.

Treatment	No.* of A. avenae/pot	No.** of A. avenae/3 g root
Control	-	-
25×10^3 A. avenae	3,418	0
50×10^3 A. avenae	3,060	0
75×10^3 A. avenae	4,478	0

* Average of 5 replicates. Each pot contained approximately 800 g of soil. The number of A. avenae per pot was estimated from 100 g soil samples.

** Root samples were stained in lactophenol-cotton blue and examined under stereoscopic mocroscope.

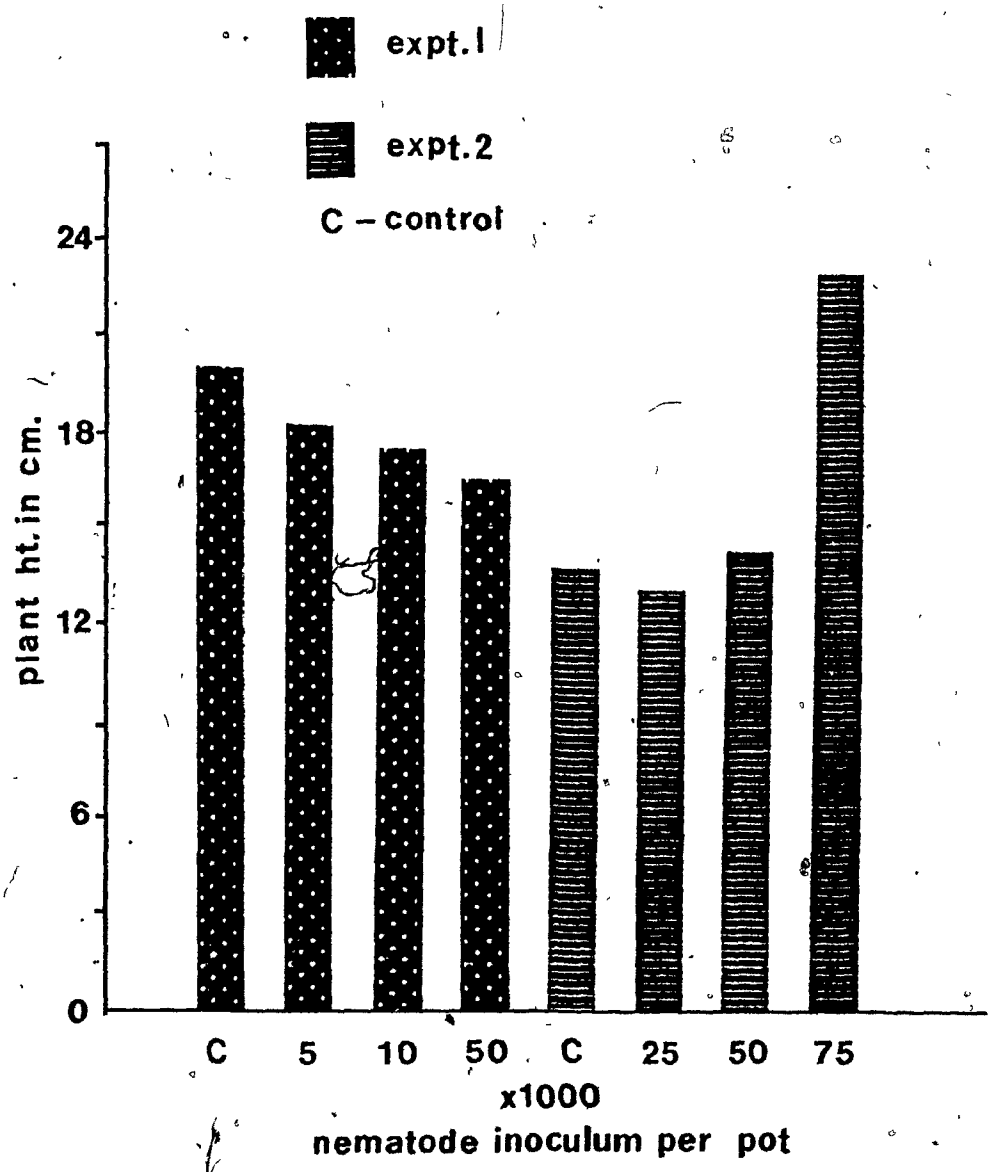

Figure 12. Height of pea plants, 30 days after soil inoculation with Aphelenchus avenae.

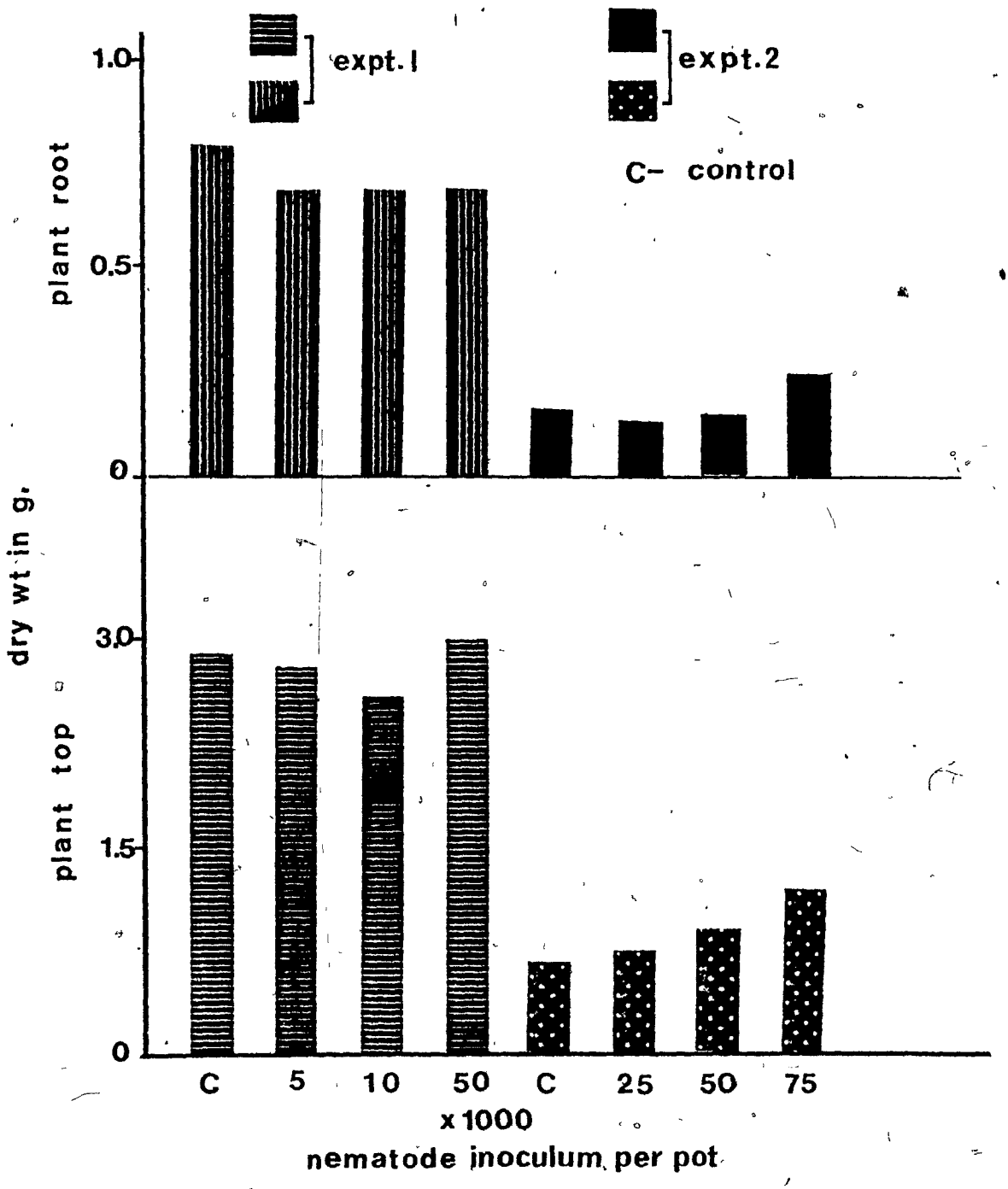

Figure 13. Dry weights of pea plants, 30 days after soil inoculation with Aphelenchus avenae.

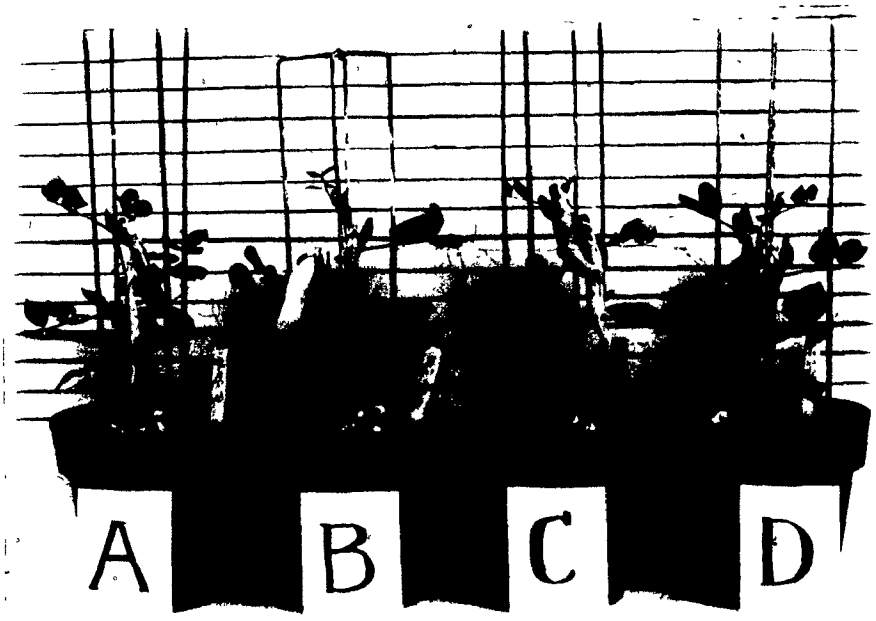

Figure 14. Pea plants grown in greenhouse, 30 days after inoculation with A. avenae. Expt. 1.
A. With $5x10^3$ A. avenae. B. With $10x10^3$ A. avenae.
C. With $50x10^3$ A. avenae. D. Control, no nematodes.

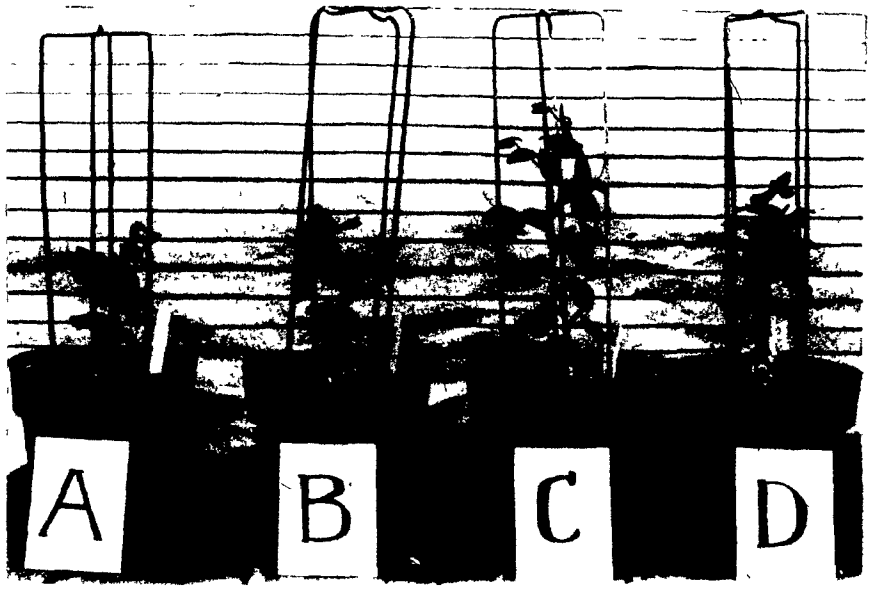

Figure 15. Pea plants grown in greenhouse, 30 days after inoculation with A. avenae. Expt. 2.
A. With $25x10^3$ A. avenae. B. With $50x10^3$ A. avenae.
C. With $75x10^3$ A. avenae. D. Control, no nematodes.

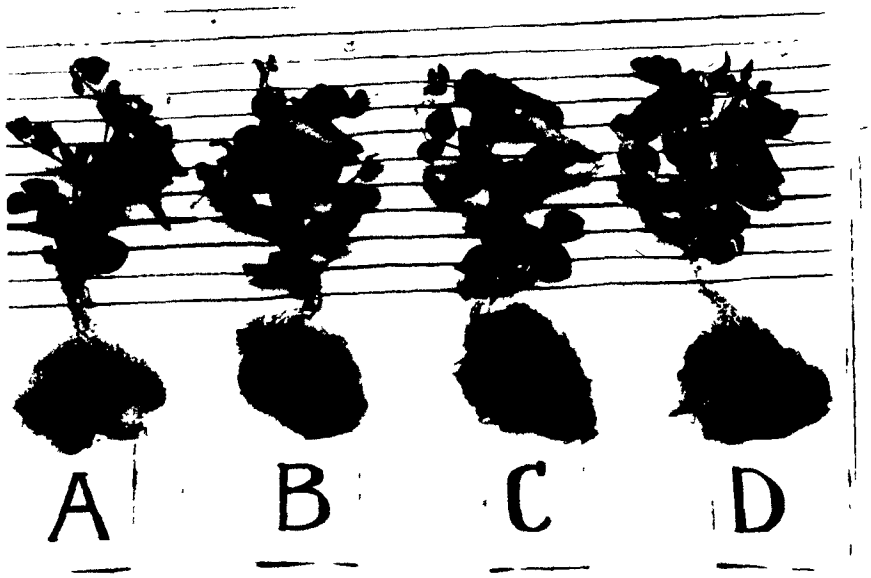

Figure 16. Pea plants harvested 30 days after soil inoculation with A. avenae. Expt. 1.
A. With $5x10^3$ A. avenae. B. With $10x10^3$ A. avenae.
C. With $50x10^3$ A. avenae. D. Control, no nematodes.

Figure 17. Pea plants harvested 30 days after soil inoculation with A. avenae. Expt. 2.
A. With $25x10^3$ A. avenae. B. With $50x10^3$ A. avenae.
C. With $75x10^3$ A. avenae. D. Control, no nematodes.

VIII. STUDIES ON THE EFFECTS OF *APHELENCHUS AVENAE* ON DAMPING-OFF OF PEA SEEDLINGS CAUSED BY *PYTHIUM ULTIMUM*

Earlier studies in this series had shown that *Pythium ultimum* isolated from local soil can cause either pre- or postemergence damping-off of pea seedlings (pages 25 and 26), and that the mycophagous nematode *A. avenae* would feed on *P. ultimum* (page 38) but not on pea plants (page 45). The potential of *A. avenae* in disease reduction had been demonstrated by several workers (Rhoades and Linford, 1959; Klink and Barker, 1968; Roy, 1973; Cheng and Tu, 1974; Porth, 1975). With this background of information it seemed logical to assume that *A. avenae* could play an important role in the control of damping-off of pea seedlings caused by *P. ultimum*. Consequently, the next series of studies had the objective of determining the effects of *A. avenae* on pre- and postemergence damping-off of pea seedlings in sterilized and nonsterilized soil under greenhouse conditions.

1. Preliminary study on the effect of *Aphelenchus avenae* on postemergence damping-off of pea seedlings in sterilized soil

A. Materials and methods

Pea seeds were surface-sterilized by the method described on page 18. One pre-soaked seed was planted per 10-cm clay pot of steam-sterilized soil.

Inocula of P. ultimum and of A. avenae were prepared by the previously described methods (pages 23 and 48). Inoculations were made when the seedlings were 12 days old (about 3-4 cm high). The fungal inoculum and the desired number of nematodes were added simultaneously to each pot. A layer of sterile fine soil was applied on top of the inoculum almost immediately after inoculation.

The experiment consisted of the following treatments, each with five replications.

Fungus alone
5×10^3 nematodes + fungus
10×10^3 nematodes + fungus
50×10^3 nematodes + fungus
50×10^3 nematodes alone
Control, no nematodes or fungus

The pots were arranged in a randomized block in the water tank which was maintained under the same conditions as stated on pate 23. The plants were watered regularly and a record was kept of the damped-off and non-damped-off seedlings throughout the experimental period. The experiment was terminated 12 days after inoculation.

After termination of the experiment, all plants in nematode or fungus-treated pots were removed, washed and stained by methods described on page 45. The presence of nematodes and fungus inside the stained roots was observed and described. Reisolation of the fungus from the infected roots was made.

B. Results and discussion

The results are summarized in Table 11. P. ultimum killed 100% of the pea seedlings where it was the only inoculum in the pots, whereas 60% of the plants survived in pots where 5,000 or 10,000 nematodes were added with the fungus, and all plants survived where 50,000 nematodes were included in the inoculum.

By examining the roots of all specimens, it was found that no nematodes had entered the roots of the plants treated with nematodes alone, but A. avenae was abundant inside the fungus-infected roots which had been inoculated with both organisms (Figure 18). It was observed that numerous resting spores of P. ultimum were produced on moribund root tissues (Figure 19B) and the fungus appeared to be in every part of the infected roots where it grew freely and produced fruiting structures (Figures 19A, 19B). P. ultimum was reisolated from the diseased plants.

From the above results, it was learned that A. avenae can suppress the damping-off disease of pea seedlings caused by P. ultimum in sterilized soil. It was felt that promotion of the survival of the seedlings was due to suppression of the activities of the fungus in the soil prior to its entry into the roots. P. ultimum sporulated abundantly in moribund root tissues where they could not be fed on by A. avenae. Such spores may have served as the main inoculum in the soil.

Table 11. Results of a preliminary study on the effect of Aphelenchus avenae on post-emergence damping-off of pea seedlings caused by Pythium ultimum. in sterilized soil.

Treatment	No. of survivors/ 5 seedlings *	% Survival
Fungus alone	0	0
5×10^3 Nematodes + Fungus	3	60
10×10^3 Nematodes + Fungus	3	60
50×10^3 Nematodes + Fungus	5	100
50×10^3 Nematodes + Fungus	5	100
Control, no nematodes or fungus	5	100

* Five replicates of one seedling per pot. Data were collected 12 days after inoculation.

Figure 18. Aphelenchus avenae in root tissues of pea infected with Pythium ultimum.

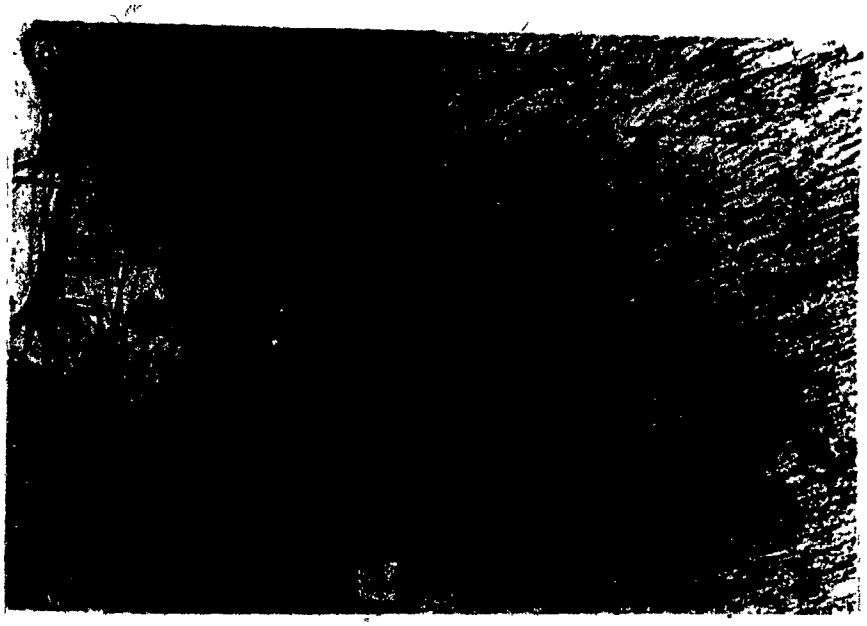

Figure 19. (A) Infection through a root hair and growth of Pythium ultimum in an infected pea root. (B) Fruiting structures of Pythium ultimum in infected root of pea.

Although Figure 19A shows P. ultimum entering a root hair, other observations indicate that this fungus can colonize and destroy the root tissues without any specific infection court or portal of entry. It may enter the plant tissues by a simple infection hypha or via an appressorium applied to the tissue prior to entry. It has been observed that P. ultimum can enter from cell junctions, epidermal cells, root hairs or root cap cells and that it grows inter- and intracellularly (Miller et al., 1966; Mellano et al., 1970; Dow and Lumsden, 1975).

2. Studies on the effect of Aphelenchus avenae on preemergence damping-off of pea seedlings caused by Pythium ultimum.

i. Experiments with sterilized soil

These experiments were carried out by adding different numbers of nematodes together with fungal inoculum to sterilized soil in pots seeded with pea seeds to determine the effect of A. avenae on preemergence damping-off of peas. At the end of the experiments, the continuing effectiveness of both the fungus and the nematodes over an additional period of 12 days was also studied.

A. Materials and methods

The surface sterilization of pea seeds and the preparation of fungal and nematode inocula were the same as previously stated, on pages 19, 23 and 48, respectively. Three seeds were planted per 10-cm clay pot containing steam-sterilized soil. Inocula of fungus and nematodes were added simultaneously after seeding, then the surface of

the inoculated pots was covered with a layer of sterilized fine soil. In control pots, only plain PDA was added instead of fungal inoculum. The pots were arranged in a randomized block in a water tank as described on page 23.

The experiments consisted of:

For experiment 1 -

Fungus alone
25 x 10^3 nematodes + fungus
50 x 10^3 nematodes + fungus
75 x 10^3 nematodes + fungus
Control, no nematodes or fungus

Experiment 2 was carried out to confirm or dispute the results of the first experiment and to extend it by adding more nematodes in one of the treatments. This experiment consisted of -

Fungus alone
50 x 10^3 nematodes + fungus
75 x 10^3 nematodes + fungus
100 x 10^3 nematodes + fungus
Control, no nematodes or fungus

The treatments were replicated five times.

The treated pots were watered regularly after inoculation and the number of emerged seedlings was recorded during the experimental period. Reisolation of the fungus was done by plating the decayed seeds on water agar. The experiments were terminated 12 days after inoculation when the total number of emerged and non-emerged seedlings was determined. The resulting data were analyzed by Kruskal-Wallis one-way analysis of variance by ranks (Siegel, 1956), for finding the differences between the treatments in which the probability was set at

the 0.05 level. This analysis method was used because these experiments contained a very small sample size.

After termination of experiment 2, all plants were removed from the pots and surface-sterilized pea seeds were planted into the same pots. This was done to determine if the nematode population was increasing or decreasing and if it was still effective in protecting pea seeds. The pots were maintained under conditions similar to the previous experiments. At the end of this third experiment, 12 days after planting, the number of emerged seeds was recorded, and the nematodes were extracted from 100-g soil samples from each pot.

B. Results and discussion

As shown in Table 12 and Figure 20, for experiment 1, all treatments with nematodes gave a highly significant effect on the percentage of emergence when compared with the check in which the percentage of emergence was zero. In experiment 2, the percentage of emergence corresponded to the increase in the number of nematodes, as shown in Table 13. There were highly significant differences (86%) between nematode-treated and nontreated seeds. P. ultimum was reisolated from randomly sampled seeds in the preemergence damped-off pots.

In experiment 1 it was found (Table 12) that treatments with the highest number of A. avenae did not give the highest percentage of emergence, whereas in experiment 2 (Table 13), all nematode treatments gave the same percentage of emergence. Moreover, in the former

experiment, the fungus alone readily killed all the seeds, whereas in the latter it did not. Perhaps these variations are due to several factors, but it was felt that fluctuations of the environmental conditions were of major importance.

The results of the third experiment, in which seeds were planted in the soils used in the previous experiment (experiment 2) are shown in Table 14 and Figure 20. It may be seen that there was a low percentage of emergence of seeds and relatively few nematodes were recovered.

Low emergence of pea seedlings from the planted pots (experiment 3) indicates that the activity of the fungus was vigorous and that of the nematodes was not, 12 days after the inoculation. It was assumed that many A. avenae died soon after lysis of the fungal hyphae occurred at the termination of experiment 2 or due to the environmental conditions, whereas the fungus survived by its "resting" structures even when the conditions in the soil became unfavorable. It is known that exudates from germinating susceptible pea seeds favor the mycelial growth and spore germination of Pythium (Flentje and Saksena, 1964; Kraft and Roberts, 1970; Short and Lacy, 1976, and others). Seeds germinating in such soil would probably stimulate the germination of these "resting" structures, so damping-off occurred while the number of A. avenae was not enough to prevent the out-spreading of the fungus.

ii. Experiment with nonsterilized soil

This experiment was carried out to determine the effect of A. avenae in natural soil and to learn how different it was from that in sterilized soil, as demonstrated in previous experiments.

A. Materials and methods

Natural soil (sandy clay, pH 7.4) was obtained from the greenhouse stock soil. It was passed through a wire sieve to remove the larger soil particles and plant debris, then it was filled into sterilized 10-cm clay pots.

Pea seeds and inocula of P. ultimum and A. avenae were prepared by the methods described on pages 18, 23 and 48. Fungal inocula and the desired number of nematodes were added soon after planting of the pea seeds. A layer of sterilized fine soil was applied on the surface of the inoculated soil. Control pots received only water instead of the plain PDA as used in previous experiments, because in natural soil PDA may help the growth of other fungi from the underlying soil or affect the growth of the seedlings.

Treated pots were maintained under the same conditions as previously described. The experiment was terminated 12 days after inoculation. Data were collected and analyzed as for the previous experiments, and the fungus was reisolated from infected seeds or seedlings.

The experiment consisted of the following treatments:

Fungus alone
50 x 10^3 nematodes + fungus
75 x 10^3 nematodes + fungus
100 x 10^3 nematodes + fungus
Control, no nematodes or fungus

Each treatment was replicated five times.

B. Results and discussion

The results of this experiment are summarized in Table 15 and Figure 20. It was found that the percentage of emergence of the seeds varied from 13 to 73%, although the highest percentage of emergence was not always related to the highest number of nematodes added per treatment. There were significant differences among the treatments.

From the results, it was felt that the germinability of the pea seeds was greatly reduced in the natural soil compared with those seeded in sterilized soil as in previous experiments. This effect may have been due to microorganisms other than P. ultimum which were in the natural soil, some of which may have been involved with P. ultimum in a disease complex. Only 80% of the seeds germinated in the nontreated pots, thus providing evidence of the influence of other organisms. This was confirmed when isolation results showed that both the introduced fungus, P. ultimum, and other fungi were in the infected seeds.

Table 12. The effect of Aphelenchus avenae on preemergence damping-off of pea seedlings caused by Pythium ultimum in sterilized soil. Expt. 1.

Treatment	No. emerged/ * 15 seeds planted	% ** emergence	
Fungus alone	0	0	a
25×10^3 Nematodes + Fungus	10	66	b
50×10^3 Nematodes + Fungus	10	66	b
75×10^3 Nematodes + Fungus	6	33	b
Control, no nematodes or fungus	15	100	

* Five replicates of three seeds per pot. Data were collected 12 days after inoculation.

** Values under a common letter are not significantly different, by Kruskal-Wallis test. ($P = 0.05$)

Table 13. The effect of *Aphelenchus avenae*, on preemergence damping-off of pea seedlings caused by *Pythium ultimum* in sterilized soil. Expt. 2.

Treatment	No. emerged/ * 15 seeds planted	% ** emergence
Fungus alone	3	20 a
50 x 10^3 Nematodes + Fungus	13	86 b
75 x 10^3 Nematodes + Fungus	13	86 b
100 x 10^3 Nematodes + Fungus	13	86 b
Control, no nematodes or fungus	15	100

* Five replicates of three seeds per pot. Data were collected 12 days after inoculation.

** Values under a common letter are not significantly different, by Kruskal-Wallis test. ($P \leq 0.05$)

Table 14. Number of emerged seedlings and number of recovered nematodes from the treated soils used in experiment 2.

Original treatment	No. emerged/ * 15 seeds planted	No. of ** nematodes recovered
Fungus alone	0	——
50 x 10^3 Nematodes + Fungus	2	405
75 x 10^3 Nematodes + Fungus	2	140
100 x 10^3 Nematodes + Fungus	8	1580
Control, no nematodes or fungus	14	——

* Five replicates of three seeds per pot. Data were collected 12 days after planting, 24 days after inoculation.

** Total of five replicates. Total number of nematodes per pot extracted 24 days after inoculation.

Table 15. The effect of *Aphelenchus avenae* on preemergence damping-off of pea seedlings caused by *Pythium ultimum* in nonsterilized soil.

Treatment	No. emerged/ 15 seeds planted *	% emergence **
Fungus alone	2	13 a
50×10^3 Nematodes + Fungus	7	46 b
75×10^3 Nematodes + Fungus	11	73 b
100×10^3 Nematodes + Fungus	9	60 b
Control, no nematodes or fungus	12	80

* Five replicates of three seeds per pot. Data were collected 12 days after inoculation.

** Values under a common letter are not significantly different, by Kruskal-Wallis test. ($P = 0.05$)

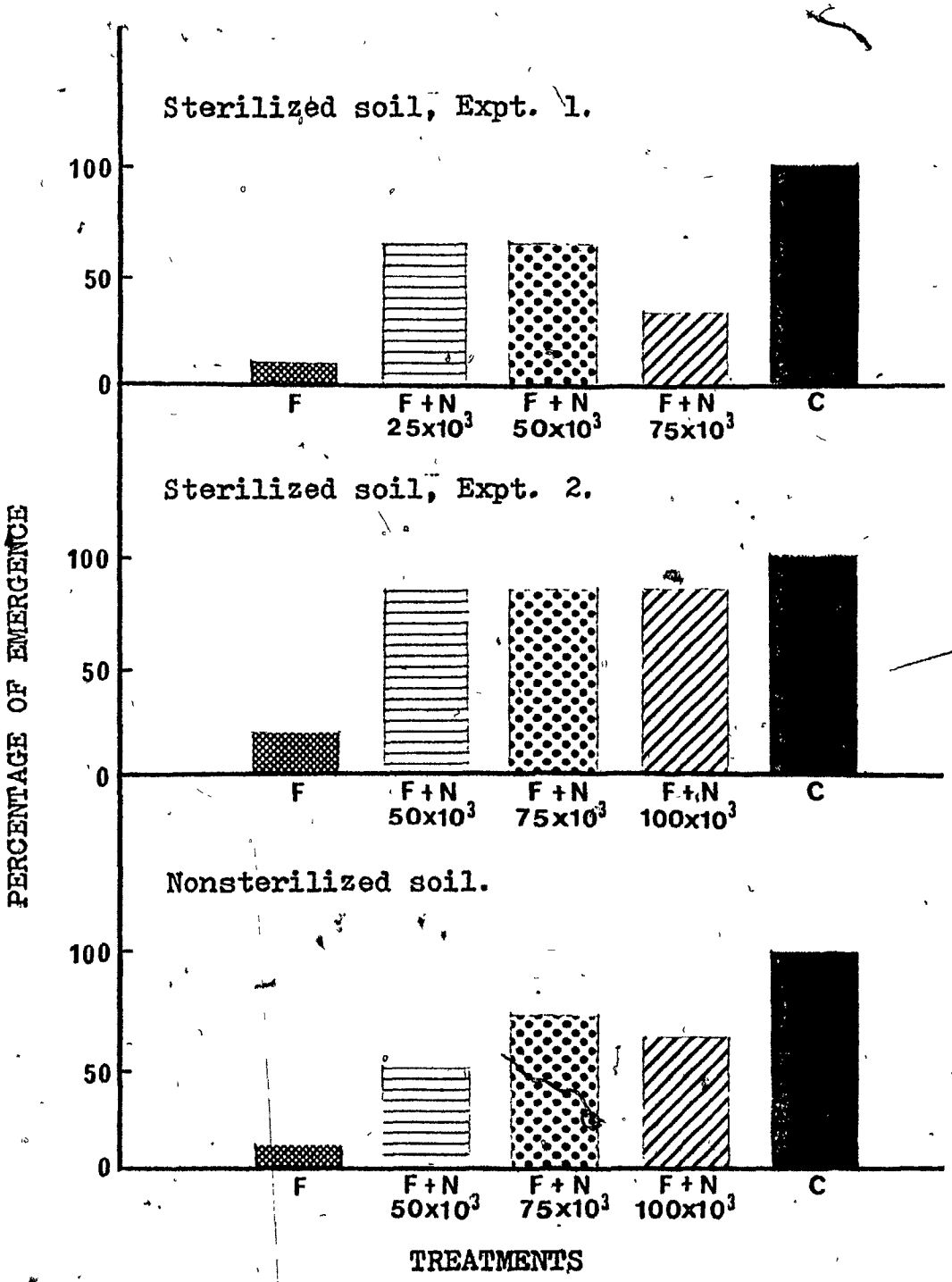

Figure 20. Effect of Aphelenchus avenae on preemergence damping-off of pea seedlings caused by Pythium ultimum.
F ---- Fungus alone
F + N ---- Fungus plus nematodes
C ---- Control, no nematodes or fungus

3. Studies on the effect of Aphelenchus avenae on postemergence damping-off of pea seedlings caused by Pythium ultimum

i. Experiments with sterilized soil

From the results obtained in previous experiments, it was shown that different population levels of A. avenae produced various effects on the incidence of preemergence damping-off of pea seedlings caused by Pythium ultimum. This study originated from the same idea but with a different aspect. In this case, effects of A. avenae on damping-off of pea seedlings were assessed at the postemergence stage.

A. Materials and Methods

The procedures used to prepare the planting materials, the inocula of P. ultimum and the nematodes were the same as previously described. Three pea seeds were seeded into each 10-cm clay pot containing steam sterilized soil. Inoculation was made when the seedlings were two weeks old (about 3-4 cm high). Fungal inocula and the desired number of nematodes were added simultaneously. Plain PDA was applied to the control pots instead of fungal inoculum. Then the pots were maintained under the conditions as stated on page 23.

The experiments consisted of:

For experiment 1 -

Fungus alone
25 x 10^3 nematodes + fungus
50 x 10^3 nematodes + fungus
75 x 10^3 nematodes + fungus
Control, no nematodes or fungus

For experiment 2 -

Fungus alone
50 x 10^3 nematodes + fungus
75 x 10^3 nematodes + fungus
100 x 10^3 nematodes + fungus
Control, no nematodes or fungus

The purposes for carrying out experiment 2 were the same as previously stated (page 66). Each treatment was replicated five times.

The number of damped-off seedlings was recorded during the experimental period and fungi were isolated from the seedlings at the termination of each experiment. Both experiments were terminated 12 days after inoculation. The data were analyzed by the same methods as used in previous experiments.

B. Results and discussion

The results of experiment 1 are presented in Table 16 and Figure 21. There were no significant differences in the treatments with 25,000, 50,000 and 75,000 nematodes plus the fungus when compared with the treatments with fungus alone. The results of experiment 2 are summarized in Table 17 and Figure 21. As in the first experiment, there were no significant differences among the treatments, although more nematodes were added in these treatments than in the previous one. P. ultimum was isolated from all specimens sampled from both experiments 1 and 2.

In the above two experiments, the overall percentage of surviving pea seedlings was very low, and the survival percentage did not conform to the numbers of nematodes added. Although there were no

statistically significant differences in the results of experiment 1, the treatment with 75,000 nematodes did produce a survival rate of 80%. However, in the second experiment this number of nematodes gave only a 40% survival rate. It was felt that this experiment may have been affected somewhat by the more adverse environmental conditions for the pea seedlings.

ii. Experiment with nonsterilized soil

The aim of this study was the same as that of the experiment introduced on page 69, except that emphasis here was on the effect of A. avenae on postemergence damping-off of the pea seedlings. The continuing effectiveness of both the fungus and the nematodes after termination of the experiment was also assessed.

A. Materials and methods

Natural soil was prepared as described on page 69, and preparations of planting materials, inocula of P. ultimum and A. avenae followed the methods described on pages 18, 23 and 48. Three pea seedlings were grown in each 10-cm pot for two weeks, then the inocula of both fungus and nematodes were introduced simultaneously. Treated pots were maintained under the same conditions as those of the previous experiments (page 23). The experiment was terminated 12 days after inoculation and data were collected and analyzed as for the previously described experiments. Random reisolations of the fungus were made during the experimental period.

After the termination of the experiment, surface-sterilized, pre-soaked pea seeds were planted into these treated soils in the same pots, after all old plants had been removed. The pots were kept under the same conditions, and were watered regularly. The emergence of the seeds was recorded and the experiment was terminated 12 days after the seeds were planted.

The treatments were as follows:

Fungus alone
50 x 10^3 nematodes + fungus
75 x 10^3 nematodes + fungus
100 x 10^3 nematodes + fungus
Control, no nematodes or fungus

Each treatment was replicated five times.

B. Results and discussion

A tabular summary of the results is presented in Table 18 and graphically by Figure 21.

Statistical analysis of the data demonstrated that there were significant differences among all of the treatments. Only 13% of the seedlings survived where no nematodes were added, whereas stands in nematodes-added pots were from 66 to 73%. P. ultimum was reisolated from the diseased plants.

From the results obtained in this experiment, it was found that the survival of the seedlings was mainly dependent on the number of nematodes added. Although the number of survivors did not correspond to the number of nematodes added to the pots, the benefit obtained by the addition of the nematodes was readily apparent in all cases.

Table 16. The effect of Aphelenchus avenae on postemergence damping-off of pea seedlings caused by Pythium ultimum in sterilized soil. Expt. 1.

Treatment	No. of survivors/ 15 seedlings *	% Survival **
Fungus alone	4	26 a
25 x 10^3 Nematodes + Fungus	7	46 a
50 x 10^3 Nematodes + Fungus	7	46 a
75 x 10^3 Nematodes + Fungus	12	80 a
Control, no nematodes or fungus	15	100

* Five replicates of three seedlings per pot. Data were collected 12 days after inoculation.

** Values under a common letter are not significantly different, by Kruskal-Wallis test. (P= 0.05)

Table 17. The effect of Aphelenchus avenae on postemergence damping-off of pea seedlings caused by Pythium ultimum in sterilized soil. Expt. 2.

Treatment	No. of survivors/* 15 seedlings	% ** Survival
Fungus alone	1	6 a
50 x 10^3 Nematodes + Fungus	6	40 a
75 x 10^3 Nematodes + Fungus	6	40 a
100 x 10^3 Nematodes + Fungus	8	53 a
Control, no nematodes or fungus	15	100

\# Five replicates of three seedlings per pot. Data were collected 12 days after inoculation.

** Values under a common letter are not significantly different, by Kruskal-Wallis test. (P= 0.05)

Table 18. The effect of *Aphelenchus avenae* on postemergence damping-off of pea seedlings caused by *Pythium ultimum* in nonsterilized soil.

Treatment	No. of survivors/ 15 seedlings *	% Survival **
Fungus alone	2	13 a
50 x 10^3 Nematodes + Fungus	10	66 b
75 x 10^3 Nematodes + Fungus	11	73 b
100 x 10^3 Nematodes + Fungus	10	66 b
Control, no nematodes or fungus	15	100

* Five replicates of three seedlings per pot. Data were collected 12 days after inoculation.

** Values under a common letter are not significantly different, by Kruskal-Wallis test. (P = 0.05)

Table 19. Number of emerged seedlings in nonsterilized soil treated with Aphelenchus avenae and Pythium ultimum in experiment 2.

Original treatment	No. emerged/ * 20 seeds planted	% ** emergence
Fungus alone	4	20 a
50 x 10^3 Nematodes + Fungus	6	30 a
75 x 10^3 Nematodes + Fungus	8	40 a
100 x 10^3 Nematodes + Fungus	7	35 a
Control, no nematodes or fungus	20	100

* Five replicates of four seeds per pot. Data were collected 12 days after planting, which was 24 days after the original soil treatments.

** Values under a common letter are not significantly different, by Kruskal-Wallis test. (P = 0.05)

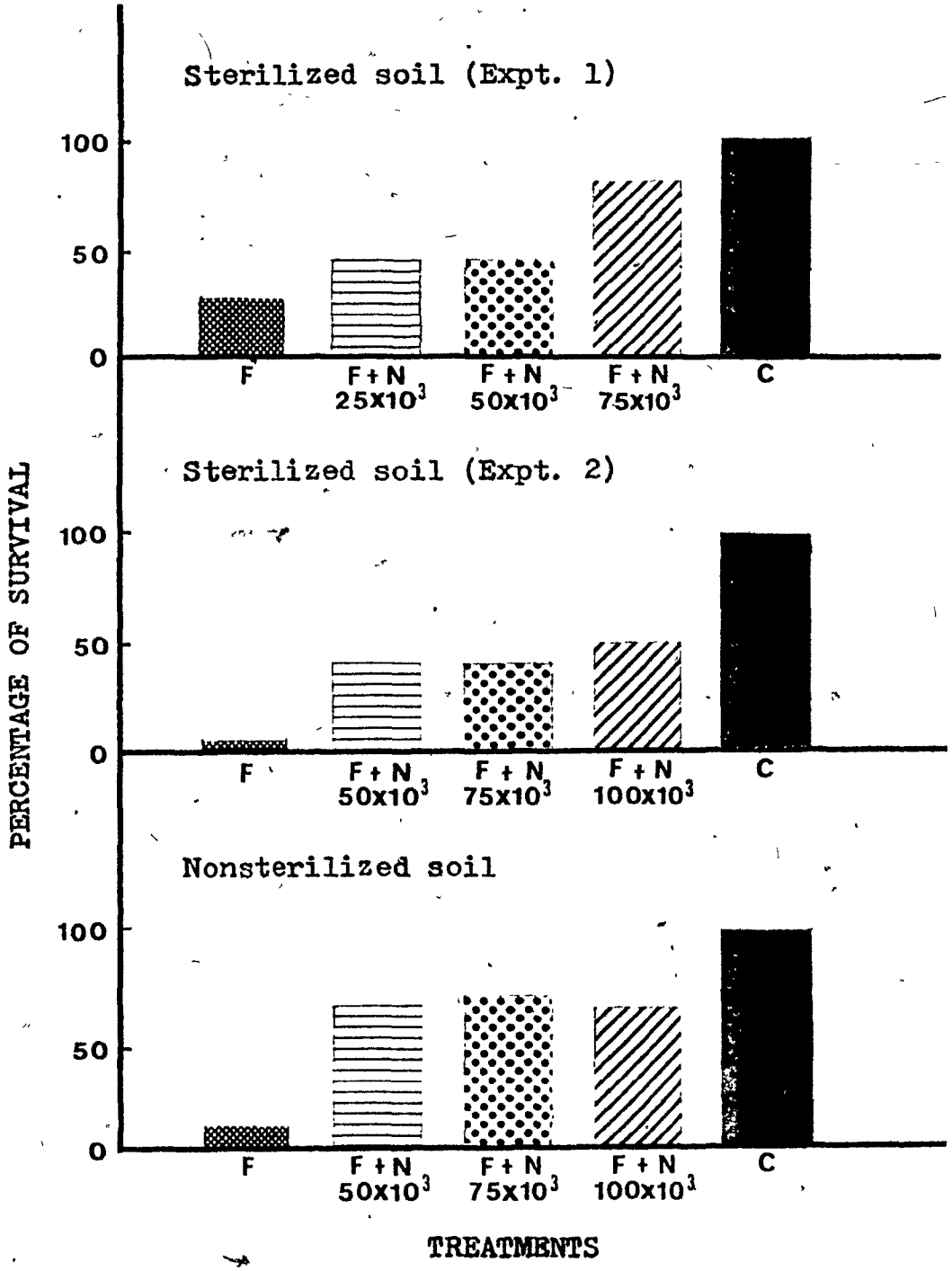

Figure 21. Effect of Aphelenchus avenae on postemergence damping-off of pea seedlings caused by Pythium ultimum.

F ---- Fungus alone

F + N ---- Fungus plus nematodes

C ---- control, no nematodes or fungus

The number of seedlings to emerge from the secondary planted seeds (experiment 3) was generally low in all nematode-treated pots (Table 19). It was obvious that the emergence of the pea seedlings was seriously affected by the P. ultimum. Indeed, many of the seeds were killed in the soil. This may have been due to the relatively low numbers of A. avenae remaining in the soil, or to influences of other microorganisms in the soil acting synergistically with P. ultimum to the detriment of the germinating seeds.

4. The effect of Aphelenchus avenae compared with a chemical drench on preemergence damping-off of pea seedlings in sterilized soil

This study was carried out to compare the effectiveness of A. avenae with that of a chemical drenching method in the control of the preemergence damping-off disease of pea.

A. Materials and methods

A damping-off control chemical "No-Damp" (Plant Products Co., Ltd., Ontario) was used in this experiment. The recommended concentration of the compound is 3 teaspoons (approximately 13 ml) in 1.135 litres of water. Two concentrations of this compound, namely, the recommended concentration and double the recommended concentration, were prepared according to the manufacturer's instructions.

Planting materials, fungal and nematode inocula were prepared by the methods previously described. For treatments with nematodes and the fungus, inoculum of P. ultimum and 75,000 nematodes per pot were added soon after seeding. Each pot was planted with three seeds. For

treatments with the chemical solutions, the inoculum of P. ultimum was first added to each pot of soil, which was then drenched with 300 ml of the desired solution. Three pea seeds were then planted three hours after drenching. A thin layer of sterilized fine soil was applied to each pot to cover the inocula and seeds.

The treated pots were maintained under the same conditions as mentioned previously. The number of emerged seedlings was recorded and reisolation of the fungus was made from the damped-off seeds. The experiment was terminated 12 days after inoculation. The data were collected and processed by the methods outlined for previous experiments.

The experiment consisted of four treatments and each treatment was replicated five times.

The treatments were as follows:

Fungus alone
No-Damp at recommended concentration + fungus
No-Damp at double the recommended concentration + fungus
75,000 nematodes + fungus
Control, no nematodes, fungus or chemical

B. Results and discussion

A tabular summary of the results is presented in Table 20.

The results reveal that there were no significant differences between the chemical treatments and the fungus alone treatments, whereas the treatment with 75,000 nematodes plus fungus gave slightly better emergence of the seedlings. There was a significant difference between this treatment and the fungus alone treatments.

With these results, it was difficult to conclude whether A. avenae would be consistently better than the compound used. However, it was again observed that A. avenae could reduce the incidence of damping-off of pea seedlings. In this instance, it provided better control than either of the two dosage levels of the commercial compound. P. ultimum was consistently isolated from damped-off seedlings in chemical-treated pots. It was felt that the compound was relatively ineffective in this test. Moreover, in the chemical-treated soil a few plants were observed to produce tiny and yellowish leaves. This was thought to be caused by the toxicity of the chemical at the two concentrations used.

Table 20. The effect of Aphelenchus avenae, compared with a chemical drench on preemergence damping-off of pea seedlings caused by Pythium ultimum in sterilized soil.

Treatment	No. emerged/ ** 15 seeds planted	% *** emergence
Fungus alone	2	13 a
"No-Damp" conc. 1 * + Fungus	5	33 ab
"No-Damp" conc. 2 * + Fungus	8	53 ab
75×10^3 Nematodes + Fungus	13	86 b
Control, no nematodes, fungus or chemical	15	100

* Recommended concentration of "No-Damp" : Approximately 13 ml in 1.135 litres of water.

Concentration 1 : Recommended concentration.
Concentration 2 : Double strength.

** Five replicates of three seeds per pot. Data were collected 12 days after inoculation.

*** Values under a common letter are not significantly different, by Kruskal-Wallis test. ($P = 0.05$)

IX. GENERAL DISCUSSION

The results obtained in this study demonstrated that Pythium species are common in local soils. By pathogenicity tests, many isolates, notably P. ultimum, were found to be highly pathogenic to pea and cucumber seedlings. The results also confirm other reports which have stated that P. ultimum is one of the most common species of the Pythiaceae to be found in North America (Middleton, 1943) and that it is one of the most destructive pathogens of peas (MacNeill, 1965; Escobar et al., 1967; Kraft and Burke, 1971). Since it causes pre- and postemergence damping-off of pea and probably other plant seedlings, it was felt that P. ultimum can be a destructive fungus in this area whenever the environmental conditions are favorable for disease development.

The reports of Mankau and Mankau (1963) indicated that A. avenae multiplied rapidly on many phytopathogenic fungi but that it did not multiply well on Pythium species. However, Rhoades and Linford (1959) observed the feeding of A. avenae on P. arrhenomanes. They reported that A. avenae controlled root-rot of corn caused by that fungus under greenhouse conditions. Chin (1964) also found that A. avenae could multiply on P. debaryanum.

In the present study, A. avenae was found to feed and multiply on several isolates of Pythium, although the rate of multiplication was not as high as that on species of Fusarium or Rhizoctonia which have frequently been reported as good hosts. Observations of the feeding of A. avenae on hyphae and allantoid bodies or frustrated appressoria of Pythium ultimum were similar to those of Rhoades and Linford (1959). The loss of protoplasm by the fungus during and after being fed on by nematodes must affect its survival and growth in soil.

The status of A. avenae as a parasite is still uncertain, though it has frequently been found in association with fungal-diseased roots and it is known to be capable of penetrating the roots of vascular plants (Chin and Estey, 1966; Terry, 1966; Klink and Barker, 1968). Barker and Darling (1965) reported that A. avenae was capable of living and multiplying on non-differentiated plant calluses.

By testing A. avenae on both axenic cultured and pot-grown pea seedlings under controlled conditions, it was found that the culture used did not penetrate the tissues of healthy seedlings. Although there were slight effects (not significant, see Appendices I-X) on growth of the pot-grown seedlings, the failure to find A. avenae in healthy root tissues strengthens the hypothesis that it is not a parasitic nematode and that it is incapable of entering or feeding upon intact, healthy tissues of pea plants. This hypothesis was also supported by the finding of very low populations of nematodes in both the soil extract culture medium and in soil in which pea seedlings were growing.

The chief aim of this study was to determine the effects of A. avenae on damping-off of pea seedlings in the soil under greenhouse conditions. The significance of A. avenae in the reduction of certain other root diseases has been demonstrated by several workers. The number of A. avenae used by these workers varied very much according to the type of diseases they studied, on the host plant used, and on the experimental conditions. They had showed that there is a tendency to obtain control of diseases caused by fungi that are good hosts for the nematode rather than those which are not. Barker (1964) and Riffle (1973) mentioned that the effectiveness of A. avenae may also be influenced by environmental conditions as well as the timing of inoculation.

In the present experiments, it was consistently found that whenever from 25,000 to 100,000 A. avenae were added to soil infected with P. ultimum, there was some reduction of the incidence of damping-off incited by that fungus. However, it was also observed that in some cases the percentage of emergence and survival of pea seedlings did not increase as the number of nematodes was increased. Such variations strongly suggest that environmental conditions play an important role in this form of biological control of a root disease. For example, the conditions which favor the activity of A. avenae (moist soil, mild temperature, etc.) also tend to favor the attack by P. ultimum. Probably, the attack by P. ultimum may also be promoted by exudates from the seeds or roots which have no known effect on A. avenae.

Suppression of damping-off by the nematodes was more pronounced in the preemergence stage than in the postemergence stage, and more effective in sterilized soil than in nonsterilized soil. In the former, it can be explained that much of the fungus was destroyed before the seeds had germinated, whereas in the latter, the plant stems or roots were more directly exposed to the fungal inoculum, thus allowing the fungus to attack soon after the inoculation.

A. avenae presumably survived and was at least partly dependent upon the hyphae of P. ultimum when added simultaneously with it into the soil. Although P. ultimum sporulated rapidly on moribund root tissues, A. avenae is incapable of feeding on these spores, therefore the nematodes would soon have a shortage of food materials. On the other hand, the spores of P. ultimum would persist in the soil to serve as inoculum for the infection of new seedlings (pages 68, 73, 79, 83). This is probably the reason why germination of seeds planted in previously treated soil was very low. These seeds were probably invaded by hyphae from the germinated spores. From the results of these experiments, there is the strong indication that A. avenae must be constantly present in the soil and in relatively large populations to retard the spread of the fungus.

The finding that A. avenae did not enter or feed on healthy root tissues of pea seedlings indicates that the nematodes were not involved in the disease complex. In contrast, A. avenae was found to migrate from soil to infected stems and leaves of damped-off plants which had

fallen to the soil surface. This finding strengthens the hypothesis that these nematodes were attracted by the fungal hyphae in or on infected tissues.

By the present test, it was found that A. avenae gave slightly better control of damping-off caused by P. ultimum than either of two concentrations of a commercial compound "No-Damp."

The findings of this study agreed with the conclusion of other workers who have stated that A. avenae is a beneficial nematode. Although the population levels necessary to give a high degree of disease control are not usually found under field conditions, the rhizospheric environment offers many opportunities for nematode-fungus interrelationships. A. avenae occurs generally in natural soils and has the ability to feed on a wide range of fungi, therefore there can be little doubt that it plays a significant role in the ecology of soil fungi.

X. SUMMARY

1. Twelve cultures of Pythium were isolated from local soil, ten of which were found to be highly pathogenic to seedlings of pea and cucumber in laboratory and greenhouse tests.

2. The isolate that killed 100% of the pea seedlings in a pathogenicity test was selected for further study. It was later identified as Pythium ultimum Trow. This fungus was found to support the reproduction of Aphelenchus avenae, which was seen to feed on its hyphae and allantoid bodies or frustrated appressoria.

3. Failure of A. avenae to penetrate or to feed on pea seedling roots in axenic cultures or in soil indicates that A. avenae is not a parasite of peas.

4. Under greenhouse conditions, in sterilized soil, populations of A. avenae from 25,000 to 100,000 per 10-cm pot of soil increased the percentage emergence and survival of pea seedlings by more than 20% when the soil was artificially infested with P. ultimum.

5. Under similar conditions, but in nonsterilized soil infested with P. ultimum, A. avenae increased the emergence and survival percentage of pea seedlings by approximately the same amount.

6. Pea seeds, which were planted into pots of soil that previously contained Pythium infected seedlings, had a very low germination percentage although the soil had been inoculated with 50,000 to 100,000 A. avenae.

7. The addition of 75,000 A. avenae to soil infested with P. ultimum gave a slightly higher emergence percentage of pea seedlings than did drenching the soil with either of two concentrations of a commercial compound recommended for the control of damping-off.

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APPENDIX TABLES I-X

ANALYSIS OF VARIANCE TABLES

TABLE I. Height of pea plants 30 days after inoculation with Aphelenchus avenae in sterilized soil - Experiment 1

Source of variation	D.F.	S.S.	M.S.	F. value	F. 5%
Total	23	148.7096			
Treatments	3	40.9646	13.6549	2.5346 ns	3.10
Error	20	107.7450	5.3873		

ns Not significant at p = 0.05

TABLE II. Fresh weight of above ground parts of pea plants, 30 days after inoculation with Aphelenchus avenae in sterilized soil - Expt. 1

Source of variation	D.F.	S.S.	M.S.	F. value	F. 5%
Total	23	355.6184			
Treatments	3	47.3484	15.7828	1.024 ns	3.10
Error	20	308.2700	15.4135		

ns Not significant at p = 0.05

TABLE III. Fresh weight of root of pea plants, 30 days after inoculation with Aphelenchus avenae in sterilized soil - Experiment 1

Source of variation	D.F.	S.S.	M.S.	F. value	F. 5%
Total	23	142.9983			
Treatments	3	13.7083	6.5694	0.7068 ns	3.10
Error	20	129.2900	6.4645		

ns Not significant at p = 0.05

TABLE IV. Dry weight of above ground parts of pea plants, 30 days after inoculation with Aphelenchus avenae in sterilized soil - Experiment 1

Source of variation	D.F.	S.S.	M.S.	F. value	F. 5%
Total	23	11.0516			
Treatments	3	0.6907	0.2302	0.4444 ns	3.10
Error	20	10.3609	0.5180		

ns Not significant at p = 0.05

TABLE V. Dry weight of root of pea plants, 30 days after inoculation with Aphelenchus avenae in sterilized soil - Experiment 1

Source of variation	D.F.	S.S.	M.S.	F. value	F. 5%
Total	23	0.4656			
Treatments	3	0.0442	0.0147	0.6977 ns	3.10
Error	20	0.4214	0.0211		

ns Not significant at p = 0.05

TABLE VI. Height of pea plants, 30 days after inoculation with Aphelenchus avenae in sterilized soil - Experiment 2

Source of variation	D.F.	S.S.	M.S.	F. value	F. 5%
Total	19	590.1080			
Treatments	3	329.1520	109.7173	6.7271*	3.24
Error	16	260.9560	16.3098		

* Significant at p = 0.05

TABLE VII. Fresh weight of above ground parts of pea plants, 30 days after inoculation with Aphelenchus avenae in sterilized soil - Expt.2

Source of variation	D.F.	S.S.	M.S.	F. value	F. 5%
Total	19	78.9620			
Treatments	3	55.2190	18.4063	12.4040*	3.24
Error	16	23.7430	1.4839		

* Significant at p = 0.05

TABLE VIII. Fresh weight of root of pea plants, 30 days after inoculation with Aphelenchus avenae in sterilized soil - Experiment 2

Source of variation	D.F.	S.S.	M.S.	F. value	F. 5%
Total	19	50.0244			
Treatments	3	28.3034	9.4345	6.9494*	3.24
Error	16	21.7210	1.3576		

* Significant at p = 0.05

TABLE IX. Dry weight of above ground parts of pea plants, 30 days after inoculation with Aphelenchus avenae in sterilized soil - Experiment 2

Source of variation	D.F.	S.S.	M.S.	F. value	F. 5%
Total	19	0.9666			
Treatments	3	0.6528	0.2176	11.1021*	3.24
Error	16	0.3138	0.0196		

* Significant at p = 0.05

TABLE X. Dry weight of root of pea plants, 30 days after inoculation with Aphelenchus avenae in sterilized soil - Experiment 2

Source of variation	D.F.	S.S.	M.S.	F. value	F. 5%
Total	19	0.1263			
Treatments	3	0.0578	0.0193	4.4884*	3.24
Error	16	0.0685	0.0043		

* Significant at p = 0.05