STUDIES ON THE BIOLOGY AND FEEDING HABITS OF SELECTED NEMATODES FROM THE RHIZOSPHERE OF LEGUMES

by

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SUPPLEMENTARY DOCUMENTS

(b) Short form

BIOLOGY OF NEMATODES FROM RHIZOSPHERE OF LEGUMES

ABSTRACT

Ph.D. R. B. Porth Plant Pathology

STUDIES ON THE BIOLOGY AND
FEEDING HABITS OF SELECTED
NEMATODES FROM THE RHIZOSPHERE OF LEGUMES

Effects of rhizobia, yeasts and temperature on saprozoic nematode growth and egg hatch, and nematode transport of rhizobia, were determined. Nematodes reproduced on several Rhizobium spp. and yeasts, in monoxenic culture. Egg-hatching increased with temperature from 15°C to 30°C. Nematodes transported R. phaseoli from non-rhizosphere to rhizosphere areas.

Influence of fungi on preferential selection and reproduction of Aphelenchus avenae, and effect of this nematode on nodulation and damping-off was studied. More nematodes aggregated in colonies of certain fungi than others. Fecundity was greatest on Fusarium sp., Fusarium culmorum and Verticillium albo-atrum. Nematodes suppressed nodulation of excised bean roots and controlled damping-off.

Effects of bean cultivar, fungi and systemic chemicals on galling and development of Meloidogyne hapla, were investigated. None of the cultivars were resistant to M. hapla. Yeasts inhibited nematode development while other fungi stimulated galling and egg-hatching. Nodulation was increased by M. hapla. Foliar and soil applied Systox and Dimethyl Sulfoxide reduced galling.

RESUME

Ph.D. R.B. Porth Phytopathologie

ETUDES SUR LA BIOLOGIE ET LES MOEURS ALIMENTAIRES D'UNE SELECTION DE NEMATODES DE LA RHIZOSPHERE DES LEGUMINEUSES

Les effets des Rhizobia, des levures et de la température sur la croissance et l'éclosion des oeufs de nématodes saprozoïtes, sont étudiés, ainsi que le transport des Rhizobia par ces nématodes. Ceux-ci se reproduisent en culture monoxène avec plusieurs espèces de Rhizobium et de levures. L'éclosion des oeufs augmente lorsque la température passe de 15°C à 30°C. Ces nématodes transportent Rhizobium phaseoli des régions hors de la rhizosphère à la rhizosphère.

L'action des champignons sur la sélection préférentielle et la reproduction de Aphelenchus avenae, et l'effet de ce nématode sur la formation des nodules symbiotiques et la fonte des semis sont étudiés. Ce nématode s'amasse en plus nombre autour des colonies de certains champignons que d'autres. La plus grande fécondité s'observe en présence de Fusarium sp., Fusarium culmorum et Verticillium albo-atrum. Ce nématode supprime la nodisité chez les racines isolées et réduit la fonte des semis.

L'influence des champignons, des composés systémiques, et de différentes variétés cultivées du haricots sur la nématocécidie et le développement de Meloidogyne Hapla a fait l'objet de notre recherche. Aucune des variétés du haricot est résistante à M. hapla. Les levures inhibent le développement du nématode, tandis que d'autres champignons stimulent la nématocécidie et l'écolosion des oeufs. La nodosité symbiotique est accrue par ce nématode. L'application de Systox et du sulfoxide de diméthyle sur le feuillage et sur le sol réduit la nématocécidie.

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GENERAL INTRODUCTION

The soil solution surrounding roots of leguminous plants contains a relatively large and complex community of organisms, including bacteria of the genus Rhizobium. Little is known of the roles being played by nematodes in the rhizosphere biota, not only in the development of legumes but in the nodulation of their roots. Because of the agricultural importance of legumes in atmospheric nitrogen fixation, a large part of this work has been devoted to a study of interactions between legumes, nodule bacteria and nematodes. Also, the growth and development of freeliving nematodes on certain members of the rhizosphere was studied.

Some of the interrelationships of Aphelenchus avenae with various soil fungi including plant pathogens, and with legume root nodule bacteria, have been included in this study.

Since Meloidogyne hapla is recognized for its destructive attacks on agricultural and horticultural plants, attempts were made to control this parasite using a systemic insecticide having nematicidal properties.

GENERAL LITERATURE REVIEW

1. The Rhizosphere of Leguminous Plants

a. Importance of the Rhizosphere

The extension of growing plant roots in soil has a marked influence on the microflora and microfauna immediately adjacent to the root surface. Hiltner (1904, cited by Garrett, 1960) was the first person to observe that microorganisms were more abundant near root surfaces than in distant soil and he defined the zone under the influence of plant roots as the rhizosphere.

Since Hiltner's pioneering work, a considerable number of papers have been devoted to the study of microorganisms and living roots. Two zones of root influence upon soil microorganisms have subsequently been characterized : (i) the root surface or rhizoplane (Clark, 1949), and (ii) the rhizosphere.

Generally, three kinds of relationships between soil microorganisms and roots of higher plants are discernible: (i) rhizosphere and root surface phenomena (microbes occur in high numbers around or on the root); (ii) parasitic (microbes invade the living root cells and debilitate the host plant); (iii) symbiotic (microbes invade the root cells and benefit the host plant, e.g. mycorrhizae, legume nodules.)

Since adequate surveys and discussion of the numerous rhizosphere studies have been presented in several reviews (Starkey, 1958; Katznelson et al., 1948; Clark, 1949; Krasilnikov, 1958; Lochead, 1959; Katznelson, 1961; Katznelson, 1965;

Rovira, 1965a,b; Parkinson, 1967; Rovira and McDougall, 1967), the purpose of this introduction is to describe the influence of the rhizosphere phenomenon on the important microbial groups found in soil.

(i) Bacteria

The early rhizosphere studies were predominantly concerned with the responses of bacteria within the sphere of influence of plant roots (Hiltner, 1904 (cited by Garrett, 1960); Starkey, 1929 a,b,c). Gradually, studies of other microbes were completed and the original concept was extended considerably to include fungi, actinomycetes, algae, protozoa and nematodes.

(ii) Fungi

The responses of fungi to the influence of the rhizosphere are less well known than those of the bacteria, primarily because the attention given to the study of rhizosphere fungi has been considerably less than that accorded to the bacteria (Parkinson, 1967). The consensus of most workers is that fungi are stimulated by plant roots, but to a lesser degree than are bacteria. This generalization is not shared by all workers; indeed, the view has been expressed that plant roots do not alter appreciably the total counts of fungi (Alexander, 1961). The above general conclusion that fungi are stimulated to a lesser degree by plant roots than are bacteria must be examined carefully. It should be noted that the most commonly used

method in counting rhizosphere fungi has been the dilution plating technique. This method permits an estimation of the numbers of rapidly growing and spore-forming types of fungi (Warcup, 1960) but neglects the slower growing and predominantly mycelial forms. Methods for assessing amounts of mycelial development in soil and rhizospheres have been developed, which, if applied, may change the above mentioned concept (Parkinson, 1967). There is good reason to support Parkinson's hypothesis as Katznelson (1965) has pointed out that one strand of fungus mycelium may be equivalent in weight and metabolic activity to many bacteria. In fact, Rovira (1965a) has speculated that the rhizosphere effect, on the basis of cell volume or cell mass, might even favor fungi over bacteria.

(iii) Actinomycetes

Despite recognition of their widespread occurrence in soil (Alexander, 1961) and importance in the cycling of soil nutrients (Kuster, 1967), actinomycetes have received much less attention than bacteria in relation to the rhizosphere (Katznelson, 1965). Where they have been studied, the majority of investigations have dwelt predominantly on their antimicrobial potentialities, especially against root pathogenic organisms and nitrogen cycle bacteria such as Azotobacter and Rhizobium (Gray and Williams, 1971; Katznelson, 1965). Although limited in number, these studies have shown that the roots of many plant types favour the development of greater numbers of

antibacterial and antifungal actinomycetes compared with root-free soil.

(iv) Algae

Information concerning the influence of plant roots on soil algae is confined to a small number of studies (Katznelson, 1965). There are indications of a range of relationships between plant roots and algae. A few workers have observed enhanced growth of algae in the rhizosphere while others have not (Lund, 1967). Unfortunately the Cyanophyta (blue-green algae) are often included in algal counts with the Chlorophyta (green algae), despite great differences in structure and physiology between the two groups.

(v) Protozoa

The rhizosphere effect on protozoa has been studied by several authors, and, in general, greater numbers of amoebae, flagellates and ciliates have been isolated from rhizosphere soil than from non-rhizosphere soil (Stout and Heal, 1967; Darbyshire and Greaves, 1973). Presumably there is a correlation between increased protozoan activity and the number of bacteria and other microorganisms serving as sources of food in the rhizosphere (Stout and Heal, 1967).

(vi) Nematodes

According to the few relevant studies published, rhizosphere soil also contains appreciably greater numbers of nematodes than root-free soil (Katznelson, 1965). Both plant

parasitic and nonparasitic forms are influenced although the freeliving nonparasitic nematodes have received very little attention in rhizosphere investigations. Jones (1959) is of the opinion that the accumulation of plant parasitic nematodes at the root-soil interface is due to the supply of organic substances in this zone, whereas plant roots do not have any specific attracting ability for the non-plant parasitic nematodes. Henderson and Katznelson (1961) and Rouatt et al., (1963) found that oats, barley, wheat, soybean and pea rhizospheres were occupied by a broad spectrum of soil nematodes with greater numbers occurring in rhizosphere soil suspensions of peas, wheat and soybeans than in those from oats and barley.

b. Microbiology of the Legume Rhizosphere

The importance of fixation of atmospheric nitrogen by complex associations of legumes and bacteria of the genus Rhizobium has been widely recognized for many years. The widespread cultivation of leguminous crops attests to the enormity of their importance in the agricultural economy of the world (Fred, Baldwin and McCoy, 1932). Figures of 100 to 200 lb per year on each acre of agricultural land under normal farm conditions represent the amount of N_2 fixed by effectively nodulated legumes (Alexander, 1961).

Thus it is not surprising to find that a great deal of interest has been shown in the symbiotic relationships of legumes and their rhizobia. The process of nodule formation,

including infection of the root hair, bacteroid formation and morphological development of the nodule are, however, beyond the scope of this literature review and will not be discussed here. General accounts of infection and the biochemistry of symbiotic nitrogen fixation are given elsewhere (Raggio and Raggio, 1962; Carnahan and Castle, 1963; Virtanen and Miettinen, 1963; Nutman, 1965; Burris, 1966).

The objective of this review is to discuss the microbiology of the legume rhizosphere. It is well known that different plant species have widely divergent numbers of organisms in their rhizospheres. And almost without exception, studies of a random series of plants, including legumes and non-legumes, have shown that legumes produce a more pronounced rhizosphere effect than non-legumes. General acceptance of the unique capacity of the legume to support larger rhizosphere populations than the non-legume is indicated in the literature (Clark, 1949; Alexander, 1961; Katznelson, 1965; Clark, 1969).

(i) Rhizosphere Effect of Legumes on Bacteria

Early investigations showed that the rhizospheres of leguminous plants afford highly favourable conditions for bacterial development, with larger numbers of bacteria occurring in the immediate vicinity of legume plant roots (Caron, 1895; Stoklasa and Ernest, 1905; Creuzberg, 1928; cited by Fred, Baldwin and McCoy, 1932) compared with non-legumes.

According to the observations of Starkey (1929b), rhizosphere counts of bacteria were greater for clover than for beets or wheat.

Graf, 1930 and Adati, 1939 (cited by Katznelson et al., 1948) noted that the increase in total numbers of bacteria was least in the case of cereals and most with legumes.

Starkey (1931) found that long lived legumes such as sweet clover exerted profound effects on the rhizosphere, yielding R:S ratios of 50 at 356 days and 217 at 437 days.

Thom and Humfeld (1932) reported a distinct increase in the number of bacteria in the vicinity of alfalfa and vetch roots compared with rye.

The rhizosphere effect of soybeans on bacteria was shown to be two times that of barley, according to R:S ratios calculated from data provided by Rouatt et al., (1960).

Similarly, R:S ratios calculated from the data of Rouatt and Katznelson (1961), revealed that red clover stimulated bacterial numbers four times that of flax, oats and wheat and seven times that of maize and barley.

Rouatt et al., (1963), noted a greater rhizosphere effect, at 85 - 90^{O}F, of soybean on bacterial numbers than with wheat. As the temperature decreased the wheat plants were seen to stimulate greater numbers of bacteria around their roots than soybeans, illustrating the effects of temperature on the rhizosphere population.

The greater abundance of ammonifying bacteria in clover rhizospheres compared to timothy was reported by Shilova and Kondrateva (1955).

Bacteria resistant to streptomycin were preferentially stimulated in the rhizospheres of 17 plant species tested but most markedly with legumes (Brown, 1961).

The role in plant rhizospheres of two of the best known genera of soil bacteria - Azotobacter and Rhizobium, both involved in nitrogen fixation - has been extensively studied. Investigations into the comparative effects of legume and non-legume rhizospheres on Azotobacter have been limited, however, and conflicting evidence has been presented. Beijerinck (1908, cited by Fred, Baldwin and McCoy, 1932) found greater numbers of Azotobacter in soil around leguminous plants than non-legumes. Krasilnikov (1958) reported a number of examples of Azotobacter stimulation in the root region of clover, alfalfa and peas whereas growth was much less on wheat, corn, flax and cotton. Contrasting evidence, nevertheless, was presented by Katznelson and Strzelczyk (1961) who demonstrated that, of 17 crop plants tested, counts of Azotobacter were similar in legume and non-legume rhizospheres.

Stimulation of species of Rhizobium in the legume rhizosphere has been known for some time (Fred, Baldwin and McCoy, 1932). Although the exact cause of this stimulation is not known, the importance of certain root exudates was first shown by West (1939) who observed that biotin and thiamine, excreted

by flax and tobacco, were stimulatory to R. trifolii. A year later, West and Wilson (1940) discovered the requirement of rhizobia for biotin. Rovira (1955, 1962) found that root exudates were more varied and abundant from legumes than from other plants. The stimulatory effect of red clover on R. trifolii was recorded by Rovira (1961) who reported R:S ratios ranging from 8 to 200 for red clover and only 6 to 13 for a grass. The enormity of population development was noted by Purchase and Nutman (1957) who counted rhizosphere populations of 10^6 to 10^9 Rhizobium spp. per ml of rhizosphere soil. Notably, legumes have been found to stimulate nodule bacteria to a greater extent than other rhizosphere microorganisms (Nutman, 1965). Nutman concluded that R:S ratios of Rhizobium in the rhizosphere are rarely smaller than 10^2 and often exceed 10^6, whereas ratios for other rhizosphere bacteria usually fall within the range of 10 to 10^2, rarely exceeding 10^3.

(ii) Rhizosphere Effect of Legumes on Fungi

Despite the filamentous nature of fungi and the prolific sporulation of certain species, which make this group more difficult to quantitatively assess than bacteria (Rovira, 1965a) most studies show a stronger rhizosphere effect by leguminous plants than non-legumes.

Starkey (1931) was the first to show that filamentous fungi were more abundant on legumes than non-legumes. He recorded an R:S ratio of 10 for sweet clover.

Peterson (1958), however, found larger numbers of fungi on and around the roots of wheat than red clover. Peterson noted, though, that realistic comparison of the two crops was questionable due to the rate of development of the wheat plants which had reached a more advanced state of maturity than the clover plants.

Comparing the number of fungi in the rhizospheres of barley and soybean, Rouatt et al., (1960) recorded R:S ratios of 2.2 for barley and 3.6 for soybean, indicating the enhanced effect of the legume (R:S ratios were calculated from data provided by the above authors).

In an extensive rhizosphere and rhizoplane study of the microflora of several cultivated plants, Ordin (1961) observed that the legume rhizosphere effect was not significantly greater than that of non-legumes. At the same time, Rovira (1961) showed that the numbers of sporing fungi in the rhizosphere zone of red clover were 3 to 4 times greater than in the rhizosphere of the grass Paspalum.

Evidence presented by Rouatt et al., (1963) demonstrated appreciably larger numbers of mycelial forms of fungi on the roots of soybeans than wheat.

While most of the studies of fungi associated with roots have been carried out on the hyphal and sporing forms, evidence from only a few studies has shown clearly that numbers of yeasts also increase in the rhizosphere (Babeva and Belyanin, 1966).

In an earlier study of yeasts in the rhizosphere of plants, Babeva and Saveleva (1963) reported that of eleven crop plants, clover had the third highest R:S ratio, indicating the effectiveness of legumes in stimulating the growth of yeasts.

(iii) Rhizosphere Effect of Legumes on Actinomycetes

Little attention has been given to the influence of plant type on the actinomycete population of the rhizosphere. Starkey (1931) demonstrated the stimulatory effect of legumes on rhizosphere actinomycetes, reporting an R:S ratio of 8.6 for sweet clover at 437 days of growth. He found that young plants, such as bean, exerted a slight effect, while mangel, beets and corn showed no influence.

(iv) Rhizosphere Effect of Legumes on Algae

There are conflicting reports on the influence of legume plant roots on algae; some authors claim stimulation, and others no stimulation.

Starkey (1958) stated that algae are not affected and may even be less numerous in the rhizosphere than in the soil; nevertheless, he did not support his statement with references or data.

Shtina (1954) reported that numbers of algae in the rhizospheres of clover, lupins and timothy were similar to those in the surrounding soil. Lupins and clover, however, were shown to harbour a high population of green algae compared with

timothy, rye and potatoes.

In later work, Shtina (1957) found that the cultivation of prolific, long-lived legume root systems led to the build up of green algae around their roots.

Under gnotobiotic conditions, pea roots were observed to stimulate profuse growth of a mixture of algae (Cullimore and Woodbine, 1963). Since the above work involved aseptic conditions, direct comparison with roots growing in soil in the presence of a mixed microflora cannot be made.

(v) Rhizosphere Effect of Legumes on Protozoa

As already mentioned, greater numbers of protozoa are found in the rhizosphere than in root-free soil.

Shilova and Kondrateva (1955) recorded a larger number of protozoa in the rhizosphere of clover than timothy.

In a detailed study of protozoa in the rhizosphere, Darbyshire and Greaves (1967) noted that more falgellates were stimulated in the rhizospheres of white clover than in the rhizospheres of either white mustard or perennial rye grass. On the other hand, numbers of amoebae were greater around roots of white mustard than the clover or rye grass.

(vi) Rhizosphere Effect of Legumes on Saprozoic Nematodes

Freeliving nonparasitic nematodes are also more abundant in the rhizosphere than in the soil around it (Katznelson, 1965). Few studies have been conducted, though, on the influence of

plant type on the freeliving nematode population of the soil.

Henderson and Katznelson (1961) observed the highest total numbers of nonparasitic nematodes in the rhizosphere of peas, and lowest in that of oats. R:S ratios calculated from their data are 13.5, 27.5, 30.9, 60.1 and 70.8 for oats, barley, soybeans, wheat and peas, respectively.

Many more Acrobeloides, Cephalobus and Dorylaimus were counted in rhizosphere soil suspensions of soybean than of wheat (Rouatt et al., 1963).

2. The Biology of Saprozoic Nematodes in the Plant Rhizosphere

A survey of the literature indicates that many different terms are used to describe the non-plant parasitic nematodes. Several of these terms such as microbial feeders (Banage, 1963), saprozoic (Jensen, 1967), freeliving (Nielsen, 1949b), saprophagous (Kozlowska and Domurat, 1971), microherbivorous (Yeates, 1971, saprobiotic (Soloveva, 1965) and microphagous (Cooper and Van Gundy, 1970) are used in a general sense to include all forms of non-plant parasitic nematodes. Others are more specific: for example, the words bacteriophagous and mycophagous (Cooper and Van Gundy, 1970) represent forms which feed on bacteria and fungi respectively. Predators are species of nematodes which feed on other, relatively large, organisms such as protozoa, nematodes and rotifers (Nielsen, 1967). Although

attempts have been made to classify the soil nematodes on the basis of food source (Nielsen, 1949b; Banage, 1963; Yeates, 1971) and thereby consolidate the above terminology, general acceptance has not been achieved. For this reason, therefore, several of the terms described above will be used throughout this literature review and study.

a. Microbial Food Sources for Saprozoic Nematodes

The kind of microorganism fed upon by saprozoic soil nematodes is related to the type of feeding apparatus possessed by the nematode in question. The majority of spear-bearing nematodes obtain their food from parasitism of higher plants. There are several species, however, belonging primarily to the order Dorylaimida, which also possess piercing spears but are thought to feed on microorganisms such as algae, protozoa or other nematodes. This latter group, while commonly included in the freeliving category since plant roots are not considered to be a primary food source, will not be considered here. Another group of freeliving nematodes which will not be included in this literature review are those which possess strongly developed tooth-like structures. These soil nematodes obtain their nutriment through predation on invertebrates such as protozoa or other nematodes. Interestingly, however, some of these predatory forms can be maintained on bacteria for several weeks (Nielsen, 1949b).

For the purposes of this literature review, therefore, only those soil nematodes belonging to the order Rhabditida and characterized by a buccal capsule, the stoma of which does not contain a spear or a tooth, will be discussed.

(i) Bacteria

Early studies on the feeding habits of saprozoic soil nematodes, which were based primarily on microscopic visualization of the gut contents, indicated that bacteria were a primary source of food. Despite these observations, the utilization of bacteria as a food source by saprozoic nematodes was not proven until 1946 by Briggs (discussed in Dougherty and Calhoun, 1948). Briggs succeeded in freeing the eggs of Rhabditis elegans from associated bacteria, transferring the eggs to agar media containing single species of bacteria and successfully building up large nematode populations.

Essentially the same conclusion was reached many years earlier by McCoy (1929) who cultured larvae of an animal parasitic nematode (Ancylostomum caninum) monoxenically with several different bacteria. McCoy concluded that living bacteria were the essential food utilized by hook-worm larvae developing to the infective stage.

Dougherty and Calhoun (1948) were able to culture 9 species of saprozoic nematodes on Pseudomonas fluorescens and Escherichia coli.

A year later, Nielsen (1949b) published his extensive studies on freeliving nematodes, which included an investigation into the growth and development of 37 species of freeliving soil nematodes on different bacteria using monoxenic hanging drop cultures.

Stock cultures of Caenorhabditis elegans and Rhabditis anomala were kept for periods of 2 and 4 years respectively on the bacterium E. coli by Nicholas et al., (1959).

Sohlenius (1968) found that several different bacteria supported the continued growth and reproduction of two species of freeliving nematodes.

Other workers successful in maintaining monoxenic cultures of freeliving nematodes includes: Chantanao and Jensen (1969); Lee et al., (1970); Tietjen et al., (1970); Yeates (1970); Smerda et al., (1971) and Palmisano and Turchetti (1972).

Studies on the suitability of various bacteria as food for freeliving nematodes were first begun by McCoy (1929) who observed that for successful monoxenic rearing of the soil-dwelling, preparasitic stages of the dog hook-worm, Ancylostomum caninum, several species of bacteria, notably gram negative rod-shaped forms, could serve.

In extensive work with Rhabditis elegans, Briggs (1946, cited by Dougherty and Calhoun, 1948) demonstrated that this nematode could be maintained on a number of gram negative rod-shaped bacteria and with one gram positive bacillus, but not

with most bacilli or with other gram positive bacteria such as streptococci or staphylococci.

In light of the work by McCoy (1929) and Briggs (1946) above, Dougherty and Calhoun (1948) concluded that the gram negative rod-shaped bacteria were the most suitable food for rhabditid forms.

Later, Sohlenius (1968) studied the effect of different bacteria upon growth rate and reproduction of Diplogaster nudicapitatus and Rhabditis maupasi, and, in support of Dougherty and Calhoun (1948) above, found that only one of three gram positive bacteria supported dense populations of both nematodes whereas all four gram negative bacteria promoted prolific nematode reproduction.

Using tracer studies, Tietjen et al., (1970) calculated the consumption of different bacteria by the freeliving marine nematode Rhabditis marina. With few exceptions, their results clearly showed that the largest amounts of bacteria ingested were from two gram negative bacteria, Flavobacterium and Pseudomonas, whereas comparatively low numbers of gram positive bacteria were consumed.

Evidence of a contrasting nature to the above generalization has been published. In studying the development of several freeliving nematodes monoxenically in depression slides with pure culture suspensions of various bacteria, Nielsen (1949b) noted the effectiveness of a gram positive Micrococcus sp. in

supporting the maturation of nematodes from eggs to the adult stage. Nevertheless, it should be pointed out that Nielsen's objective was maturation of a single generation in contrast to previous and following examples of sustained nematode cultivation.

Maintenance of six dune sand nematode species in monoxenic culture with the gram positive bacterium Bacillus cereus var. mycoides was reported by Yeates (1970).

Palmisano and Turchetti (1972) cultured Mesodiplogaster lheritieri on two gram positive bacteria, Bacillus subtilis and B. megaterium, under monoxenic cultural conditions. It was noteworthy that growth of the nematode population was slower and maximal density lower, than values reported by other workers for rhabditid nematodes on gram negative bacteria.

The effect of agriculturally important bacteria such as the root nodule organism was included by Nielsen (1949b) in his extensive study on the role of different bacteria and other microorganisms in the nutrition of saprophagous nematodes. Nielsen found that a mixture of unidentified bacteria from root nodules of Trifolium, one of which presumably was Rhizobium trifolii, supported growth, to the adult stage, of larvae of Plectus granulosus, P. parvus and Rhabditis monhystera. Other than Nielsen's synxenic study above, no investigations have been made on the effectiveness of different species of Rhizobium in supporting the growth of freeliving nematodes under monoxenic conditions. The apparent complete absence of studies of this

type is rather curious since the widespread occurrence of rhizobia and freeliving nematodes in soil is well known.

(ii) Fungi

The visualization of fungal spores and mycelia in the oesophageal lumen or intestinal tract of rhabditid nematodes has raised the question of the extent to which ingestion of these fungal structures occurs. In an early study, Cobb (1924) noted that two species of Rhabditis contained numerous spores of Trichoderma and hyphae of an unknown fungus. Both types of fungus structures were thought to provide the principal, if not the only, ingested food.

The discovery of partially digested fungus mycelia in the intestine of Plectus communis led Steiner (1927) to conclude that this form lives, at least partly, on fungus mycelia.

Nearly forty years later, and using controlled feeding conditions, Jensen (1967) observed that Diplogaster lheritieri readily ingested spores of several different fungi including Fusarium oxysporum f. lycopersici, Verticillium dahliae, Geotrichum sp., Hormodendrum sp., Penicillium sp., and Stysanus sp., but not Alternaria dauci or Ascochyta pisi. Spores of certain fungi such as V. dahliae almost completely occupied the intestine, whereas others, notably Geotrichum sp. occurred only occasionally. Although strands of unidentified hyphae were occassionally observed in the intestine, Jensen claimed that fungal spores and bacterial contaminants were more common.

Jensen was also able to demonstrate the passage of ingested spores of several fungi through the digestive tract and excretion, in a viable state, onto a plate of agar. The excreted spores were allowed to germinate and later identified.

Soloveva (1965) noted the presence of spores of Plasmodiophora brassicae inside the intestine of a saprobiotic nematode, Panagrolaimus rigidus.

Jensen and Siemer (1969) concluded that Mesodiplogaster lheritieri not only ingested spores of two plant parasitic fungi, but protected them against certain fungicides and various concentrations of chlorine.

Yeates (1970) found that freeliving nematodes isolated from dune sands carried fungal spores in their intestinal tracts.

Further evidence concerning the capability of fungus spore ingestion by freeliving nematodes was provided by Barron (1970) who showed that relatively large numbers of conidia of Harposporium helicoides, a soil-borne nematophagous hyphomycete, were rapidly consumed by a Rhabditis sp. Due to their parasitic nature, the fungal structures did not serve as a food source to the nematode.

The above examples indicate the apparent frequency and ease with which freeliving nematodes ingest the spores of certain terrestrial fungi. To date, however, no statements other than those of Cobb (1924) and Steiner (1928) above, have been made

concerning the nutritional importance of the ingested fungi. Monoxenic cultures of rhabditids with single species of fungi have not been successful.

Conflicting evidence concerning the culture of freeliving nematodes on yeasts, which taxonomists consider to be fungi, has been presented in the literature.

McCoy (1929) concluded that the yeast Torula rosea would not support cultivation of larvae of Ancylostomum caninum.

Although Nielsen (1949b) observed large numbers of cells of a colourless yeast in the saprozoic nematode Alaimus primitivus, he was unable to culture Rhabditis monhystera on the yeast Cryptococcus.

Feeding and reproduction of the marine nematodes, Metoncholaimus sp. and Acanthonchus cobbi on marine yeasts was reported by Hopper and Meyers (1966,a,b). It is quite likely, though, that bacteria carried as contaminants by the above nematodes, developed along with the yeast cells and were utilized by the rapidly developing nematodes.

The culturing of Panagrellus silusiae on agar-grown yeasts was reported by Yuen (1968). Again the growth of contaminating bacteria can be suspected as details regarding the preservation of monoxenic conditions were not given by Yuen.

b. Effect of Temperature on Saprozoic Nematode Egg Hatch

Little is known about the influence of specific environmental factors, such as temperature, on egg hatch of freeliving nematodes. Although Nielsen's (1949b) comprehensive study of freeliving soil nematodes did not include the effects of temperature on egg hatch, the importance of temperature on the rate of reproduction was acknowledged by Nielsen to be significant.

In a study of Paroigolaimella berensis and Fictor anchicoprophaga, Pillai and Taylor (1968) found that eggs of P. berensis hatched between 10 and 32°C whereas F. anchicoprophaga hatched between 10 and 38°C. Egg death occurred at 35°C for P. berensis and 38°C for F. anchicoprophaga. The time required for egg hatch at specific temperatures (10, 15, 20, 25, 30, and 35°C) between the above ranges was similar for both nematodes. Pillai and Taylor further noted that at 10°C the hatched juveniles remained viable but did not reach maturity.

Sohlenius (1968) observed that the upper limit of hatching for Rhabditis terricola was between 28.5 and 30°C. Sohlenius was also able to demonstrate egg production by R. terricola and Mesodiplogaster sp. at 5°C. Although hatching did not occur at this temperature, eggs transferred to 25°C hatched and development was normal.

Yeates (1970) determined the effect of temperature on egg hatch of eggs produced during the lifespan of six dune sand

nematodes cultured monoxenically on Bacillus cereus var. mycoides. With the exception of one nematode, percent egg hatch was greater at 20°C than 15°C (percentages were calculated from the data of Yeates).

c. Biological Role of Saprozoic Nematodes in Soil

The extensive studies of Nielsen (1949b) show that appreciable numbers and kinds of freeliving nematodes occur in soil. Although distribution of these organisms in various soils is uneven (Winslow, 1960), greatest numbers are always found in areas of organic decay (Croll and Smith, 1970; Sohlenius, 1973). From a survey of the literature, which will be discussed below, the role of soil-borne freeliving nematodes appears to be two-fold: (1) consumption and (2) dissemination of soil micro-organisms, including the plant pathogens.

(i) Consumption of Soil Microorganisms

One of the major functions of saprozoic nematodes in soil involves ingestion and utilization of various microorganisms as sources of food. Microscopic gut content analysis of several Rhabditid species isolated from soil has revealed that these nematodes often ingest large numbers of algae (Nielsen, 1949b; Leake and Jensen, 1970; Tietjen et al., 1970), fungi (Cobb, 1924; Jensen, 1967) and bacteria (Nielsen, 1949b). Evidence from feeding studies also indicates a selectivity toward certain microbial groups. Indeed, because of the relative ease with which freeliving nematodes can be monoxenically cultured with

many bacteria (Nielsen, 1949b; Dougherty and Calhoun, 1948) and the heretofore unsuccessful attempts to culture these nematodes under monoxenic conditions with algae (Tietjen et al., 1970) or fungi (Jensen, 1967), it is highly probable that bacteria are the major food source for saprozoic soil nematodes. This is in agreement with other workers (Nielsen, 1949b; Winslow, 1960; Nicholas and Jantunen, 1966; Sohlenius, 1968; Yeates, 1970) who have suggested that bacteria appear to be the primary food source for freeliving soil nematodes.

Not all bacterial genera are suitable food sources. Several pure culture studies reveal that some bacteria will not support nematode growth (McCoy, 1929; Briggs (1946, cited by Dougherty and Calhoun, 1948); Nielsen, 1949b; Sohlenius, 1968; Tietjen et al., 1970). This may be due to: (1) formation of toxic substances (Sohlenius, 1968), (2) lack of certain nutrients required at vitamin level (Tietjen et al., 1970), (3) structural reasons such as large cellular aggregates too massive to be ingested by the nematode (Nielsen, 1949b), or (4) absence of enzymes required by the nematode to digest a broad spectrum of bacterial forms (Tietjen et al., 1970). Inasmuch as there is some evidence concerning the kinds of bacteria nematodes will ingest and utilize for growth, it is interesting to note that nothing is known of what they digest and assimilate (Yeates, 1971). Furthermore, since the majority of studies concerning the diet

of the freeliving nematode have been conducted using agar plate cultures, the role of bacteria in the natural diet is really unknown (Yeates, 1971).

One of several factors influencing the growth of soil bacteria, and indirectly, the growth of freeliving nematodes, is the plant root system. Although Jones (1959) stated that plant roots do not produce specific attracting substances for non-plant parasitic nematodes, evidence exists for an indirect mechanism involving attraction and growth of saprozoic nematodes in plant rhizospheres. In 1961 Henderson and Katznelson postulated that population growth of certain parasitic soil nematodes is directly governed in the rhizosphere by the amino acid content released by the roots in question. In later work, Katznelson and Henderson (1962) demonstrated the attractiveness of colonies of actinomycetes and fungi, normally non-food hosts, to Rhabditis oxycerca. The authors concluded that attraction of freeliving nematodes to plant roots may be due to the metabolic products of the microorganisms present, several of which may not be food sources. Katznelson and Henderson (1963) went on to show that movement of freeliving nematodes such as R. oxycerca, to the rhizosphere, may also be due to the production of ammonia by ammonifying bacteria acting on amino acids released by plant roots. Kozlowska and Domurat (1971) found great increases in the number of two forms of freeliving nematodes in the root zone of rye seedlings, with no increase in root-free soil. A

direct correlation was noted between bacterial numbers in rhizosphere and non-rhizosphere soil and nematode numbers. The above information may help to explain why certain species of freeliving nematodes predominate around the roots of certain plants (Goodey, 1963; Deubert, 1959, cited by Katznelson, 1965).

Enumeration of bacterial cells ingested by freeliving nematodes has been recorded by only three workers. Chantanao and Jensen (1969b) calculated an average of 46,000 Agrobacterium tumefaciens cells consumed by female Pristionchus lheritieri in 24 hours. They found that male P. lheritieri ingested only 10,000 cells in the same period. Tietjen et al., (1970) determined the number of cells of 14 different bacteria taken up by Rhabditis marina. Approximately 43,000,000 cells of a Pseudomonas sp. and 530,000 cells of a Flavobacterium sp. were consumed per nematode per day. Other bacteria were taken up in relatively low numbers. The number of cells of an unidentified bacterium ingested by male and female Pelodera chitwoodi, over an eight day life span, were estimated as 3,100,000 and 10,000,000 respectively (Mercer and Cairns, 1973).

The fate of ingested microorganisms includes either death in the digestive tract of the nematode, or defecation in a viable condition. Palmer and MacDonald (1974) noted that the number of propagules of Fusarium moniliforme per unit of soil were reduced by saprozoic nematodes, indicating destruction of

the fungus by the nematodes. A wide variety of microbial structures have been shown to survive passage through the alimentary tract of freeliving nematodes. These include bacterial cells (Chantanao and Jensen, 1969b; Smerda et al., 1971), mycoplasma (Jensen and Stevens, 1969), bacterial viruses (Jensen and Gilmour, 1968; Chantanao and Jensen, 1969a), fungal spores (Jensen, 1967) and algae (Leake and Jensen, 1970; Tietjen et al., 1970). Saprozoic nematodes can also ingest and protect pathogenic fungal spores against fungicides (Jensen and Siemer, 1969).

Although evidence at present is limited, it appears that the ability to survive the digestive system of freeliving nematodes depends on the amount of the available food source, the species of ingested microorganism and the type of nematode. Chantanao and Jensen (1969b) suggested that the massive growth of bacteria on nutrient agar media may exceed the digestive capacity of the freeliving nematode Pristionchus lheritieri, thereby allowing the defecation of more unharmed cells than if fewer bacteria were ingested. Of two kinds of algae consumed by P. lheritieri, viable cells of one were recovered from intestinal contents and faeces, whereas viable cells of the second type were rarely recovered (Leake and Jensen, 1970). Smerda et al., (1971) noted the sensitivity of two species of Salmonella to passage through the digestive system of P. lheritieri.

They found that S. typhi could be recovered in only 20% of the trials whereas S. wichita recovery was 93% under the same conditions. The excretion of an infectious bacteriophage by two of three freeliving nematodes was shown by Jensen and Gilmour (1968).

Not only are ingested microorganisms defecated in a normal viable condition, as indicated above, but they may also be metabolically damaged in such a way that abnormal growth patterns occur after defecation. Jensen and Stevens (1969) observed atypical colony formation by Mycoplasma gallisepticum cells after excretion by three different saprozoic nematodes.

The effect of saprozoic nematodes on population levels of soil bacteria is poorly known but is thought to be significant (Alexander, 1971), and extremely complex (Nielsen, 1967). Nielsen (1949a) suggested that the main bulk of freeliving nematodes in soil tended to reduce the number of bacteria, thereby reducing the total physiological activities of these organisms. However, the activities and numbers of grazed bacteria did not continue to decline as Nielsen (1949b) stated that an acceleration occurs in the division rate of nearby non-ingested bacteria resulting in occupation of the space previously held and an increase in the bacterial population. Data was not provided by Nielsen in support of the above generalizations. Observations made by Dougherty and Calhoun

(1948) using slant cultures, and by Sohlenius (1969) using agar plate cultures, support the above contention of Nielsen (1949a). The latter authors noted that saprozoic nematodes commonly kept the bacterial population considerably reduced. On the other hand, Yeates (1971) pointed out that bacterial densities in the natural soil environment do not approach those on nutrient supplemented agar media.

(ii) Dissemination of Soil Microorganisms

During movement through natural soil or cultures on artificial media, and subsequent ingestion of microorganisms, free-living nematodes acquire an external and internal microflora. As the nematodes travel from one microhabitat to another, adhering surface microorganisms are dislodged and digestive contents containing microbial cells are voided, thus providing a mechanism for dissemination. The significance of nematode assisted microbial dissemination in soil is observed in: (1) decomposition processes in soil, and (2) the rhizosphere.

Cobb (1924) stated that saprohagous nematodes were important dispersal agents of decay-promoting, and humus-forming, microorganisms. Transfer of a Streptomyces griseus phage by surface cuticle absorption and transmission through the digestive system of freeliving nematodes was shown by Jensen and Gilmour (1968). The authors speculated that normal dispersal of soil-borne phage is slow but may be increased by freeliving nematode

activity. Mercer and Cairns (1973) pointed out that freeliving nematodes mechanically stir organic matter and associated bacterial colonies. This results in the separation of dead cells in old colonies and simultaneously exposes these cells, containing immobilized nutrients, to extracellular digestive enzymes in the soil solution which were unable to penetrate the aged colonies prior to nematode action.

As pointed out previously, larger numbers of freeliving nematodes occur in rhizosphere zones than in root-free soil. By inference, therefore, it is reasonable to suspect that these rhizosphere nematodes are very active agents of microbial dissemination. With the exception of one article, though, there is almost a complete lack of information on the interrelationships between saprozoic nematodes and dispersal of the rhizosphere microflora, despite several recorded observations of their cohabitation of the same environment. Bonifacio and Marinari (1970) found that surface sterilized saprozoic nematodes were able to multiply on voided bacteria which were deposited around the roots of wheat seedlings. The authors concluded that dissemination of the internal microflora assured survival of the nematodes.

Several studies pertaining to interaction between saprozoic nematodes and plant pathogenic microorganisms have been documented. Christie (1960) suggested that freeliving nematodes, through

transfer of decay bacteria and fungi into non-colonized plant tissue at the edge of plant root lesions, were important agents in enlargement of root lesions. From laboratory investigations, Jensen (1967) concluded that in plant rhizospheres, pathogens may be mobilized and directed to vulnerable sites along the roots by freeliving nematodes. Soloveva (1965) found that zoospores of Plasmodiophora brassicae were carried into the root zone of healthy cabbage plants by Panagrolaimus rigidus. Microscopic analysis revealed zoospores on the cuticle and in the intestine of the nematode. In a study on interactions between four plant pathogenic bacteria and Pristionchus lheritieri, Chantanao and Jensen (1969a) suggested that the most important role of the saprozoic nematode was one of dissemination. Upon demonstration of nematode transmission of phage particles able to lyse cells of Agrobacterium tumefaciens, an important plant pathogen, Chantanao and Jensen (1969b) claimed that saprozoic nematodes in plant rhizospheres may be of potential importance in plant disease control.

3. Interaction of Aphelenchus avenae with Soil-borne Fungi and Plant Roots

Assessment of the kinds of nematodes associated with different crops (Taylor et al., 1958; Taylor and Schleder, 1959) and the pioneering rhizosphere studies of Henderson and Katznelson

(1961) and Rouatt et al., (1963), have led to the generally accepted conclusion that Aphelenchus avenae can be located regularly in almost any sample taken from the roots of plants (Thorne, 1961; Jenkins and Taylor, 1967). Much interest has been shown in this nematode and the literature is replete with studies on its physiology, toxicology, taxonomy and interaction with the animate and inanimate environment. The literature to be reviewed herein will be concerned with four behavioral aspects of A. avenae interacting with other organisms: (1) attraction to different fungal hosts, (2) multiplication on different fungal hosts, (3) parasitism of higher plant roots, and (4) control of root diseases caused by fungi.

a. The Attractiveness of Different Fungi to A. avenae

Pertinent information relating to the ecology of freeliving and plant parasitic soil nematodes was summarized in 1960 by Winslow. At this time relatively little was known about the attractiveness of fungi for nematodes such as A. avenae. From the evidence available, Winslow (1960) concluded that A. avenae was not attracted by fungi from a distance, and that contact between the fungus and motile nematode was due only to chance.

In a study concerning the relationship between Aphelenchoides parietinus and various soil microorganisms, Katznelson and Henderson (1964) showed that Winslow's interpretation above was incorrect, and that a mycophagous nematode could indeed be

attracted from a distance to fungi. They added 0.2 ml of a heavy suspension of A. parietinus at the edge of a plate of Potato Dextrose Agar (PDA) opposite a fungal colony and observed, after 6 days at 15°C, the distribution pattern of the nematodes with reference to the fungal colony. Of 54 cultures tested, 43 were favorably attractive, whereas a number (Aspergillus niger, Myrothecium sp., Penicillium sp. and Phoma sp.) were not favorable to the nematode.

A detailed investigation of the attractiveness of 53 fungi to A. avenae in PDA culture (Townshend, 1964) demonstrated that within 18 hr at 25°C, this nematode was attracted to all fungi except Sclerotium rolfsii and Agaricus hortensis. Townshend noted that migration toward the fungal colonies occurred immediately for many of the nematodes and those that did not respond as quickly did not become attracted until migrating randomly over the agar.

In 1967, Pillai and Taylor recorded a distinct preference by five different mycophagous nematodes, including A. avenae, for certain species of fungi and showed that a fungus preferred by one nematode was not necessarily preferred by another nematode. A. avenae aggregated around Mortierella pusilla and Fusarium moniliforme in large numbers whereas Phytophthora cactorum and Polyporus sulfureus were not highly preferred by this nematode.

Foreman (1968) observed movement of four species of mycophagous nematodes towards different fungi when each species of nematode was placed 4 mm and 8 mm away from the fungal colonies. He regarded the nematode food finding process as consisting of two phases: (1) random movement until a gradient created by the fungus was reached, and (2) directional movement under the influence of the gradient.

Klink et al., (1970), using a modified version of Townshend's (1964) technique, investigated the degree of attractiveness of four fungi to Neotylenchus linfordi, as measured by the time required for the nematode to reach the different fungal colonies. Gliocladium roseum was shown to be more preferable than Pyrenochaeta terrestris, Chaetomium indicum and Rhizoctonia solani, in that order. Microscopic observation of N. linfordi movement on the same plate as G. roseum revealed, initially, arcing motions of the nematode heads as the individual nematodes circled at random. As the nematodes began to move toward the G. roseum colony, however, the length of the arc decreased, and within 1 cm of the colony, the nematodes moved quickly to the edge and commenced to feed on the outgrowing hyphae. These results are in agreement with the above observations of Foreman (1968).

Not only are mycophagous nematodes capable of preferentially selecting between fungal hosts, as evident by rapid aggregation around certain colonies, but, the fact that they respond in a similar fashion to culture filtrates indicates that

diffusible attracting agents excreted by the fungus may be involved. Foreman (1968) reported that four mycophagous nematodes congregated in different numbers about areas where fungal exudates were placed. This worker emphasized that caution be used in the interpretation of culture filtrate studies. Foreman measured the response of nematodes to two methods of fungal exudate filtration and showed that the more sensitive filtration process removed some substance(s) favourable to the nematodes, thereby resulting in little or no aggregation in areas of filtrate deposition. Klink et al., (1970) studied the response of Neotylenchus linfordi to culture filtrates of Gliocladium roseum and discovered that, inasmuch as the technique by which the nematodes were introduced and assayed affected the response of the nematode, 70 to 86% of the nematodes moved to G. roseum filtrate within 4 hr after introduction. Not all excreted fungal metabolites are stimulatory. Mankau (1969 a,b) found that exposure of A. avenae to culture filtrates of Aspergillus niger caused 100% immobilization of the nematode in 4 hr. Mankau further noted that in plates inoculated with both Rhizoctonia solani and A. niger, A. avenae survived only within areas occupied by R. solani. Additional to the role of fungal mycelia and diffusible compounds in attracting mycophagous nematodes, is the factor of pH. Townshend (1964) suggested that the repellent nature of Sclerotium rolfsii was due to the low pH around the fungus and Edmunds (1967) corroborated Townshend's speculation

by showing that A. avenae aggregated in regions with a higher pH than in areas with a pH value equal to or less than the surrounding agar.

Organisms other than fungi have been discovered to attract mycophagous nematodes. Of 56 actinomycetes tested, Katznelson and Henderson (1964) observed that 15% exerted an attraction for Aphelenchoides parietinus after 5 days incubation and 30% after 15 days. They found that certain culture filtrates were decidedly attractive to this nematode. Townshend (1964) reported the attraction of A. avenae to seedling roots of barley, oats, peas and wheat in agar culture, and, where the leaves of the seedlings had come in contact with the agar, nematode aggregation was also observed. Townshend pointed out that A. avenae was attracted more to certain fungi than to roots of the seedlings tested.

Although evidence is limited, some bacteria have been found to have a repellent effect on mycophagous nematodes. Baker et al., (1954) stated that the potato rot nematode, Ditylenchus destructor, which is capable of feeding on fungi as well as several higher plants, rapidly evacuated infected tubers exhibiting bacterial wet rot. Their results were substantiated by Faulkner and Darling (1961) who detected few D. destructor in tubers manifesting rapidly progressing bacterial wet rot. Later, Katznelson and Henderson (1964) demonstrated that pure cultures of bacteria and their culture filtrates were repellent to the mycophagous nematode Aphelenchoides parietinus. These workers

calculated that 51 of 60 bacterial isolates repulsed A. parietinus while in only one instance were nematodes observed to accumulate in and around a bacterial colony.

Attraction of mycophagous nematodes by fungi, other soil microorganisms, or plant roots probably occurs to a significant extent in the rhizosphere of plants. Katznelson and Henderson (1964) suggested that aggregation of Aphelenchoides parietinus around plant roots was due to the attractiveness of a food source such as fungi, and was regulated by the concentration of bacterial metabolites. Migration of mycophagous nematodes into the rhizosphere may also be governed by the attractiveness of the fungal flora, which is predominantly in the mycelial state (Harley and Waid, 1955), and by the attractiveness of the roots themselves (Townshend, 1964).

b. Growth and Multiplication of A. avenae on Fungi

Despite the fact that mycophagous nematodes are known to multiply on callus tissue of plants (as will be discussed later), and have been cultured axenically (Buecher et al., 1970; Hansen et al., 1970), the results of several workers have revealed that in nature their primary food source consists of the fungi.

Faulkner and Darling (1961) studied the reproduction of Ditylenchus destructor, the potato rot nematode, on 115 species of fungi isolated from soil or decaying potato tubers. They found that D. destructor fed and reproduced on 64 species of

fungi representing 40 genera, 8 orders and all classes of the higher fungi. Faulkner and Darling further noted that reproduction did not occur on Penicillium janthinellum, but developed in great numbers on P. javanicum and 8 other species belonging to this genus. No apparent reproduction was observed on Rhizoctonia solani, Volutella roseola, Stachybotrys sp., Fusarium oxysporum f. pisi race 1 and 2, Cephalosporium sp., Botrytis sp., Thielavia basicola, Penicillium variable, Aspergillus niger and 17 of 19 species of Phycomycetes.

Effective feeding and reproduction of Neotylenchus linfordi on Pyrenochaeta terrestris was detected by Hechler (1962a). On PDA, the progeny of single gravid females reached 40,000 to 70,000 in 30 days. Hechler (1962b) also showed that A. avenae could multiply on P. terrestris reaching population levels of 75,000 to 100,000 in 14 to 16 days at 28°C.

Mankau and Mankau (1962) reported extensive population development of A. avenae on several phytopathogenic soil fungi. Several Phytophthora sp. and Pythium ultimum did not support multiplication of the nematode. A number of saprophytic soil fungi appeared to produce meager populations of A. avenae.

Barker (1964) stated that reproduction of A. avenae was highly variable on different isolates of Rhizoctonia solani cultured on PDA or Czapek's medium. That different isolates of R. solani vary greatly in their capacity to support high populations of A. avenae led Barker to conclude that the rate

of multiplication of the nematode is affected by the genetic makeup of the fungus.

Using a visual estimation technique to determine population density of A. avenae, Townshend (1964) discovered that 49 of 54 different species of fungi were hosts for this nematode. Three species of Botrytis supported between 50,000 to 243,000 progeny. Five fungi, namely Aspergillus niger, Gongronella butleri, Penicillium frequentans, P. thomii and Sclerotium rolfsii were not hosts for A. avenae.

Goodey and Hooper (1965) demonstrated the fecundity of A. avenae in agar culture on mushroom mycelia. Single larvae increased within a three week period to approximately 70,000 to 90,000 progeny. Mention of two other fungi capable of supporting reproduction of this nematode was made but no data were given regarding progeny numbers.

Arrold and Blake (1966) investigated the growth and multiplication of Ditylenchus myceliophagus and Aphelenchoides composticola on malt peptone agar-cultured Agaricus bisporus. After 28 days at 25°C the mean number of D. myceliophagus varied from 108,000 to 164,000 depending on the number of nematodes in the inoculum. In comparison, only 16,000 to 28,000 A. composticola developed under the same conditions.

Although numerical data on population numbers was not provided, Chin and Estey (1966) noted rapid multiplication of A. avenae on several species of soil fungi.

The fecundity of five mycophagous nematodes, including

A. avenae, on ten different fungi was determined by Pillai and Taylor (1967). Generally, five of the fungi tested were excellent hosts for most of the nematodes, two were good hosts, two were poor, and Pythium irregulare was considered a nonhost. These workers found that A. avenae multiplied extensively on Alternaria solani, Rhizoctonia solani, Fusarium solani, F. moniliforme, Mortierella pusilla and Pyrenochaeta terrestris. On the other hand, Chaetomium globosum allowed only moderate reproduction and Pythium irregulare, Phytophthora cactorum and Polyporus sulfureus did not support growth of this nematode.

Eichenmuller (1968) pointed out that while A. avenae and Neodiplogaster myceliophagus were able to multiply on a wide variety of fungi, some fungi consistently produced larger populations than others. The most rapid nematode reproduction occurred when the nematodes were feeding on a fungus cultured on a weak medium. Both nematodes failed to reproduce on Sclerotium rolfsii, thus supporting the results of Townshend (1964).

The effect of temperature on the reproduction of four isolates of A. avenae on Rhizoctonia solani was measured by Evans and Fisher (1970a). After 10 days, there were great differences in progeny number between isolates at all temperatures used.

In a more detailed study, Evans and Fisher (1970b) presented evidence demonstrating that different fungal hosts

affect the number of A. avenae in a population. All fungi tested were hosts for the nematode. These workers found that the type of medium used to culture the fungi affected the magnitude of nematode progeny numbers. A total of 711,400 A. avenae developed on Rhizoctonia solani cultured on a special Rhizoctonia Medium (RM), whereas 576,500 developed on Potato Dextrose Agar. Evans and Fisher also determined the suitability of each fungus host for supporting the production of a given number of nematodes. Rhizoctonia solani on RM was most efficient and Verticillium albo-atrum least efficient.

Results derived from attempts to correlate the attractiveness of fungi, and the ability of these fungi to serve as hosts for mycophagous nematodes, are contradictory. Data published by Katznelson and Henderson (1964), Townshend (1964) and Pillai and Taylor (1967) indicate that fungi which are attractive to mycophagous nematodes are not necessarily the most suitable hosts for population increase. Klink et al., (1970), however, showed that the attraction of Neotylenchus linfordi to four different fungi could be correlated with the relative suitability of the fungi for reproduction of the nematode.

c. Parasitism of Roots of Higher Plants by A. avenae

Although numerous studies have shown that utilization of fungal hyphae by A. avenae for growth and development is commonplace, the conflicting results of several workers have made

the status of this nematode as a parasite of higher plants quite tenuous.

A number of reports have been published providing data which supports the parasitism concept of A. avenae on higher plants. Admitting the difficulty of ascertaining with certainty the pathogenicity of A. avenae, Christie and Arndt (1936) suggested that cells of living cotton seedlings constitute part, and probably the greater part, of the nematodes food. Steiner (1936) presented histological evidence that A. avenae not only could migrate through root (phlox hybrid) parenchyma tissue, but was capable of reproduction within the root system. As a result of observing A. avenae in adjacent cortical tissue of cotton lesions, and the stunting effect of large numbers of this nematode on cotton plants, Arndt and Christie (1937) argued that the presence of A. avenae in hypocotylar lesions would have a debilitating effect on the plants. Nielsen (1949b) noted that on several occasions, A. avenae had been found parasitizing plants. Goodey (1951) stated that A. avenae could penetrate and live in healthy plant tissues and function as a facultative parasite. Thorne (1961) reported that A. avenae frequently had been found inhabiting crown, leaf sheath, root cortex and other plant parts. This worker suggested that the nematode could feed on cellular contents of the living tissues. Considerable penetration of healthy bean roots by large populations of A.

avenae was observed by Barker (1964). Barker concluded that the nematode was not pathogenic to bean as there was no reduction in growth of plants inoculated with as many as 100,000 nematodes. Chin (1964) observed ectoparasitic feeding of A. avenae on root hairs of corn, oats, beet, turnip, cabbage and radish but did not detect any reproduction after 3 weeks. This worker noted that root hairs of several plant cultivars were not fed upon by A. avenae. Barker (1966) detected appreciable polygalacturonase and cellulase activity in A. avenae, indicating that puncturing of plant cell walls containing pectin and cellulose may occur as a result of enzyme secretion by this nematode. Chin and Estey (1966) found that, after 60 days, large populations of A. avenae caused stunting and wilting of oat and cabbage seedlings on warm days, whereas control plants remained normal throughout the test period. Terry (1966) noted the presence of nematodes and eggs of A. avenae in roots of several cultivars of corn and tomato. This worker also observed reduction of plant height in nematode treated plants compared to the uninoculated control. Klink and Barker (1968) claimed that the root systems of beans and peas were freely invaded when large populations of A. avenae were present. These workers suggested that the presence and reproduction of the nematode in the root system may enhance root deterioration through limited feeding on host tissue.

Studies showing reproduction of A. avenae in sterile plant tissue culture (Doliver et al., 1962; Barker, 1963; Chin,

1964; Barker and Darling, 1965; Sudakova and Chernyak, 1967, cited by Zuckerman, 1971) and excised roots (Sudakova et al., 1963; 1965, cited by Zuckerman, 1971) provide additonal support for the plant parasitic role of A. avenae.

Other workers consider A. avenae as a mycophagous nematode of the soil or rhizosphere environment, completely unable to reproduce on intact, healthy plant roots. Rhoades and Linford (1959) were unable to detect the presence of A. avenae in corn roots inoculated with nematodes alone. These workers admitted that A. avenae may feed ectoparasitically on plant roots but concluded that nutrient provision was not sufficient to allow reproduction of the nematode. Winslow (1960) emphasized that fungi are probably the main, if not the sole source of food for A. avenae. Mankau and Mankau (1962) failed to detect any root damage of sweet orange seedlings by A. avenae despite addition of 10,000 to 200,000 larvae and adults. The authors were unable to recover any adults or larvae after several months indicating the inability of this nematode to reproduce on roots of a higher plant. No indication of bean root entry or damage, by A. avenae, was found by Mankau and Mankau (1963). Jenkins and Taylor (1967) expressed strong reservations regarding the claim that A. avenae is a parasite of higher plants. However, the authors suggested that under certain conditions this nematode could enter plant roots and may feed parasitically. Sutherland (1967) concluded that A. avenae was unable to penetrate and

multiply on the roots of seven types of conifer seedlings. Sutherland emphasized that proof of plant parasitism by this nematode cannot be claimed by merely finding A. avenae in the tissues of higher plants. Sutherland and Fortin (1968) noted that A. avenae not only failed to enter roots of red pine but was incapable of destroying established mycorrhizae. In view of these results and since A. avenae was able to reproduce on the mycorrhizal fungus in pure culture, Sutherland and Fortin concluded that A. avenae cannot enter, feed upon, or reproduce on intact healthy tissues of higher plants, but utilizes fungi as a source of food.

d. Influence of A. avenae on Root Diseases Caused by Fungi

Because of the propensity of A. avenae for fungal mycelia, its potential for the control of fungal induced root diseases has been assessed by several workers. Schindler and Stewart (1956) observed reduction of wilt symptoms of carnation due to Fusarium oxysporum f. dianthi, when 3,200 Ditylenchus spp. were added per pot. In an extensive study of the effects of A. avenae on root rot of corn incited by Pythium arrhenomanes, Rhoades and Linford (1959) found that 125,000 nematodes per pot gave control of the disease whereas 50,000 nematodes were only slightly effective in decreasing root rot incidence. Because their results were obtained using sterilized soil, Rhoades and Linford proclaimed that determination of the significance of A. avenae

in control of fungal root rots in nature remained to be achieved. Barker (1964) studied the effect of A. avenae on disease reduction of Rhizoctonia solani on beans and showed that 100,000 nematodes per 5-inch crock gave almost complete control, 50,000 gave moderate control, and 10,000 gave only slight control. Barker concluded that under greenhouse conditions A. avenae is beneficial, therefore supporting the conclusion of Rhoades and Linford (1961). In reasoning further, however, Barker emphasized that the beneficial effects of this nematode under field conditions would be limited since the population levels necessary to give a high degree of control are normally not found in the field. Root rots of pea and bean, caused by six different fungi, were substantially reduced when populations of A. avenae were approximately 4,000 to 6,000/ml of fungus inoculum (Klink and Barker, 1968). These workers noted that higher or lower populations gave poorer root rot control. Riffle (1973) used flask culture conditions to demonstrate suppression of Armillaria mellea root rot of Pinus ponderosa by Aphelenchoides cibolensis and A. composticola.

On the other hand, experimental data has been published showing that root disease severity may increase or remain the same when A. avenae is combined with a pathogenic root infecting fungus. Christie and Arndt (1936) discovered increased damping-off of cotton seedlings when A. avenae was combined with the fungus Fusarium moniliforme. Later, these authors (Arndt and

Christie, 1937) were unable to detect any influence of A. avenae or Aphelenchoides parietinus on the amount of cotton wilt caused by Fusarium vasinfectum. Chin and Estey (1966) found that tomato seedlings inoculated with Verticillium albo-atrum and 60,000 A. avenae wilted to a greater degree than plants inoculated with V. albo-atrum alone. In a study on interaction between A. avenae and Verticillium dahliae on sunflower seedlings, Terry (1966) observed that, in combination, 40,000 A. avenae plus V. dahliae gave a higher disease percentage than plants receiving the fungus alone.

4. Interaction of Root-knot Nematodes with Legumes, Associated Microflora and Systemic Pesticides

In this review, it is not proposed to deal with the extensive literature pertaining to the physiology and biochemistry of Meloidogyne host-parasite relationships. Also, much of the detail concerning chemical control of the root-knot nematode will be omitted from this review. Instead, information will be presented on four aspects of the interaction between M. hapla and legume plants, including: (1) growth and development on different plant cultivars, (2) influence of saprophytic and parasitic soil fungi on root penetration and egg hatch, (3) effect of root-knot nematodes on interaction between Rhizobium spp. and legume hosts, and (4) use of systemic phosphates on development and control of M. hapla.

a. Growth and Development of M. hapla on Different Legume Cultivars

The effect of host cultivar on the morphological development of the root-knot nematode has received little attention. Barrons (1939) studied the nature of root-knot resistance in many plant species including resistant and susceptible cultivars of beans. He found that whereas juvenile nematodes entered susceptible and resistant bean roots in equal numbers, after several weeks of plant growth, numerous large galls had formed on the susceptible cultivar (Kentucky Wonder) whereas only a few tiny galls were found on the resistant cultivar (Alabama No. 1). In lima beans, Vigna sinensis, he observed that the cultivars Hopi 155 and Henderson Bush were equally invaded and galled, initially, but the galls on the resistant Hopi 155 did not develop beyond slight swellings while those on the susceptible cultivar Henderson Bush developed rapidly. Barrons concluded by hypothesizing that resistance to root-knot nematode development and reproduction was due to synthesis of substances by the plant which counteracted the giant cell inducing effect of the salivary excretions of the juvenile nematodes. Christie (1946), in an investigation of root-knot nematode development on several plant species, including seven bean cultivars, concurred with the results of Barrons (1939) regarding juvenile entry into resistant and susceptible plants. Christie noted that while some plants were highly suitable hosts for the root-knot nematode and others were highly unsuitable hosts, a range of

nematode development between these two extremes was normally observed for most plants. He also emphasized that the severity of galling was not necessarily related to development of the nematode since plants with consistently severe galling often contained nematodes incapable of producing eggs. In a thorough study of nematode resistance in the common bean, Blazey et al., (1964) found that 17 of 55 cultivars were resistant, but none were immune to M. incognita. These workers further noted that bean cultivars most resistant to M. incognita were heavily infested by M. hapla, M. javanica and M. arenaria thamesi. They concluded that known resistance in the common bean was probably confined to M. incognita only. Fassuliotis et al., (1970) showed that resistance in bush type snap beans to M. incognita was due to the absence of adequate giant cell development and to hypersensitive reaction within the infected portion of the root. No differences were observed by these workers in the number of juveniles penetrating susceptible or resistant lines, thus supporting the results of Barrons (1939) and Christie (1946). Some Phaseolus species are more resistant than others to certain species of Meloidogyne. Hutton et al., (1972) showed that 36 lines of Phaseolus atropurpureus tended to be quite resistant to M. arenaria, M. incognita and M. javanica, but not to M. hapla.

Reynolds et al., (1970), in studies of the development of M. incognita acrita on alfalfa, demonstrated that juveniles

entered resistant and susceptible cultivars in approximately the same numbers, but, after 7 days, most had migrated out of the resistant roots whereas in susceptible roots the nematodes became sedentary and developed normally.

The development of Meloidogyne incognita acrita on soybeans was studied by Crittenden (1958) who reported that in resistant cultivars: (1) giant cells did not form around the nematode head, (2) a small number of giant cells were produced, (3) the giant cell area was small, (4) giant cell cytoplasm was sparse and not dense, (5) few enlarged nuclei were present in each giant cell, and (6) little enlargement of the pericycle was observed. Crittenden obtained the opposite results with susceptible soybean cultivars. In an extensive study of soybean resistance to four species of Meloidogyne, Dropkin (1959) found that: (1) number and size of egg masses produced by M. hapla varied considerably between 19 soybean cultivars, (2) gall size was large for M. incognita acrita, M. incognita and M. arenaria, whereas only tiny galls were formed by M. hapla, (3) giant cells were large and smooth with uniformly stained contents and thick walls in susceptible cultivars, whereas the giant cells were small and appeared necrotic in resistant cultivars, and (4) egg production was much greater in susceptible cultivars forming large giant cells than in those resistant to the nematode. Bryant and Wyllie (1968) noted that resistant soybean cultivars developed fewer and smaller galls and supported nematode development

less well than susceptible cultivars, when inoculated with M. incognita acrita.

b. Effect of Soil Fungi on Development, Pathogenicity and Egg Hatch of Root-knot Nematodes

The concept of plant disease etiology involving associations of plants with several pathogenic microorganisms has become well established, and published evidence of these complexes is accumulating rapidly. The interrelationships of nematodes with other plant pathogens have recently been reviewed by several authors; bacteria, fungi and viruses were covered by Pitcher (1965) and Powell (1971a); bacteria and fungi by Mountain (1965); bacteria by Pitcher (1963) and fungi by Powell (1963, 1971b). This review, however, will be devoted to the examination of certain known interactions involving nematodes and non-pathogenic fungi and bacteria which, although harmless by themselves, cause severe plant disease in the presence of nematodes, especially members of the root-knot group.

Edmunds and Mai (1966a) studied the effect of alfalfa roots inoculated with Trichoderma viride and Fusarium oxysporum, on penetration by Pratylenchus penetrans. They found that greater numbers of P. penetrans could be recovered from roots treated with varying spore concentrations of both fungi, than from uninoculated roots. More P. penetrans juveniles entered T. viride infected alfalfa roots, at all four fungus inoculum concentrations, than fungus-free roots, whereas in experiments

with F. oxysporum, significantly more nematodes entered fungus-infected roots only at the higher concentrations of fungus inoculum.

In another study published the same year, Edmunds and Mai (1966b) noted that the population of P. penetrans recovered from alfalfa roots after 8 weeks was significantly greater in roots infected with a combination of P. penetrans and T. viride than in treatments with nematodes alone. This relationship was not the same with celery. No reason was given by these authors for the significant increase in nematode numbers in fungus infected plants. Edmunds and Mai also observed appreciable root and shoot fresh weight reduction in alfalfa and celery by the combined action of the two organisms. The greater attraction of P. penetrans to T. viride infected alfalfa seedlings than to healthy roots was again reported by Edmunds and Mai in 1967. They suggested that this attraction may have resulted from an affinity of nematodes for the greater quantity of CO_2 which was released from the fungus infected roots than from healthy roots.

Mayol and Bergeson (1970) showed that secondary micro-organisms, consisting of non-pathogenic bacteria and fungi, caused extensive weight reduction of foliage and roots of tomato plants inoculated with Meloidogyne incognita. Aseptically grown plants exposed to the same number of juveniles were heavily galled and exhibited reduced foliage growth and increased root growth. Galls on septically grown plants became necrotic,

yielding many bacterial colonies and a few fungi, whereas aseptic roots, although considerably galled, remained firm and free from bacteria or fungi. Bacteria which were isolated from surface sterilized galls removed from roots exposed to secondary microorganisms, were found to belong to the nutritional group associated with root rots. The fungi, although few in number, were identified as Trichoderma sp., Fusarium sp. and Rhizoctonia solani. Mayol and Bergeson concluded that secondary invasion by the rhizosphere microorganisms occurred as a result of increased host susceptibility through nematode modification of root cell structure and physiology.

Powell et al., (1971) investigated the interaction of several non-pathogenic soil-inhabiting fungi with M. incognita on tobacco. None of the fungi induced disease in the absence of M. incognita whereas root necrosis occurred when plants were subjected to M. incognita in combination with any one of the fungi. Necrosis was notably severe in treatments where nematodes preceded the fungi by several weeks. These workers hypothesized that root-knot nematodes tend to predispose certain hosts to subsequent invasion by a range of other microorganisms present in the rhizosphere. Moreover, they claimed that root-knot nematode damage to tobacco is most severe only when these pathogens are members of disease complexes.

Hatching of plant parasitic nematode eggs, and the factors which affect this process, have been reviewed by Shepherd and Clarke (1971). Little is known about hatching of nematode

species other than Heterodera. Although several authors have loosely suggested that egg hatch may be influenced by soil inhabiting microorganisms, elucidation of the role of microorganisms in egg hatch of plant parasitic nematodes is restricted by lack of direct study. Bergman and Van Duuren (1959) found that bacterial secretions may contain inhibitory substances which kill eggs within the cyst of Heterodera schachtii. Giebel (1963) studied the effect of different soil fungi, belonging to the genera Penicillium and Aspergillus, on hatching of H. rostochiensis. He concluded that microorganisms living in the rhizosphere play a role in the formation of a hatching factor which eventually stimulates the cyst-contained eggs to hatch.

c. Interrelationships Between Rhizobium spp., Plant Parasitic Nematodes and Leguminous Plants

The role, or status, of plant parasitic nematode components of legume-root nodule bacteria interactions has received attention from root disease investigators.

Jones and Moriarty (1956) were the first workers to record reduced nodule formation on legumes in the presence of parasitic nematodes. According to these workers, nitrogen fertilizer counteracted the suppressive effect of Heterodera goettingiana on pea nodulation.

Stimulation of root-knot nematodes by legume bacterial inoculum was observed by Shands and Crittenden (1957). They found, in greenhouse pot tests, that the amount of galling on

the susceptible soybean cultivar Adams, and penetration of the resistant cultivar Anderson, increased when Meloidogyne incognita acrita interacted with legume bacteria.

In assessing the role of root-knot nematodes in legume nodulation, Masefield (1958) reasoned that nematode galls impaired nodulation in two ways: (1) chemically, by utilizing plant nutrients at the expense of nodule bacteria, and (2) physically, by occupying sites normally available for nodules.

An inverse correlation between numbers of nodules and cysts on Lee soybeans was shown by Endo and Sasser (1958) in soil fumigation experiments. Plants heavily infested with cysts of Heterodera glycines were sparsely nodulated, while in treatments which reduced the cyst index, plants were heavily nodulated.

Reduced yields of soybeans were attributed by Ichinohe (1959) to inhibition of nodulation by Rhizobium japonicum in the presence of the soybean cyst nematode, H. glycines. Root nodules per unit weight of root were fewer on susceptible than resistant cultivars. Ichinohe observed that heavily nodulated plants often had the smallest number of females per unit weight of root. This indicated to Ichinohe that the bacterial component of the legume-bacterial-nematode complex was capable of inhibiting development of the nematode, thus supporting the observations of Endo and Sasser (1958).

Ross (1959) reported complete inhibition of nodulation on soybeans by heavy infestation of H. glycines.

Extensive invasion of pea, alfalfa and clover nodules by Pratylenchus globulicola, and subsequent nodule deterioration, were considered by Romaniko (1961) as primary factors in reduced yields of these leguminous plants in Russia.

The infestation of cowpea nodules and other root sites by the root-knot nematode Meloidogyne javanica, was found by Robinson (1961) to occur extensively on portions of root systems growing near the soil surface. Solitary nodules in the lower root zones were normally free from nematodes. Robinson pointed out that nodules attacked by juveniles during early stages of development often became galls, whereas when nodules were invaded at a later stage they retained the normal nodular morphology.

Epps and Chambers (1962) noted that the addition of Rhizobium japonicum inoculum to soybean seeds did not increase nodulation in soil heavily infested with Heterodera glycines. Instead, reduced nodulation occurred. Soil fumigation increased nodulation significantly, apparently through nematode destruction. Epps and Chambers claimed that nodule reduction was due to interaction of root-rot organisms and nematodes which caused rotting of the soybean roots, a loss of nodules already developing, and destruction of sites for additional nodules.

In a study on the effect of Meloidogyne incognita acrita on nodulating and non-nodulating strains of soybeans, Crittenden (1962) observed root enlargement and slow female maturation on the nodulating strain, whereas root enlargement was slight and

female nematode development rapid on the non-nodulating strain. Giant cell appearance was similar in both nodulating and non-nodulating strains. Since the nodulating soybean strain was not favorable for rapid female maturation compared with the non-nodulating strain, it is possible that the single gene for nodulation represses the development of this nematode.

Heavy galling of hairy vetch roots shortly after inoculation with Rhizobium leguminosarum, and complete inhibition of nodulation, convinced Malek and Jenkins (1964) that root-knot nematodes interfere directly with the establishment of nodule-forming bacteria. Nodulation was also inhibited greatly by two other nematodes, Trichodorus christiei and Criconemoides curvatum. Malek and Jenkins reasoned that either the root hair infection sites were destroyed, or the roots were made incompatible with the bacteria, by the feeding actions of the nematodes.

Nigh (1966) found that M. javanica reduced nodule formation in alfalfa by 50% when treatments were applied at the time of planting, and by 30% when nodule inoculation was delayed 32 days. In contrast to the results of Shands and Crittenden (1957), Nigh discovered that the amount of galling was reduced, and not increased, in the presence of Rhizobium.

Besides reduced root nodulation and nitrogen fixation, Ross (1969) showed that Heterodera glycines caused soybean yield reduction which was greatest when nematode-infected soybeans became increasingly deficient in nitrogen.

In a critical study of the effects of nematodes on legume

nodulation, Taha and Raski (1969) noted that Meloidogyne javanica and Heterodera trifolii infection did not significantly reduce the number of nodules per gram of white clover root, although the nematodes reproduced readily in nodular tissues. They pointed out that nodule reduction was due to reduced root growth and not the direct effect of the nematode treatment.

Panayi (1970) reported decreased nodulation in roots of beans inoculated with Rhizobium phaseoli and Meloidogyne hapla. This worker did not observe nodule invasion using 15 eggmasses as inoculum, but with 100 eggmasses added simultaneously with rhizobia, nodules were invaded by nematodes.

Barker et al., (1971) observed that the number of cysts of Heterodera glycines was reduced by the simultaneous inoculation of soybean roots and Rhizobium japonicum. Since nematode hatch, penetration and cyst development on soybean were reduced by applications of $NaNO_3$ or NH_4NO_3, these workers concluded that the reduced numbers of cysts could have been due to the inhibitory effects of nitrate on juvenile hatch or emergence.

Races of the soybean cyst nematode were found to differ quantitatively not only in their influence on nodulation but also on nitrogen fixation and nodule efficiency (Lehman et al., 1971). Race 1 of H. glycines, together with R. japonicum, caused a significant decrease in nodules per gram of root tissue and nitrogen-fixing capacity per plant as compared with R. japonicum control plants. Races 2 and 4, however, at the same inoculum densities as race 1, did not decrease nodules per gram

of root tissue or the nitrogen-fixing capacity per plant. Race 1, but not races 2 and 4, caused severe chlorosis of soybean.

Reduction in bacterial nodulation of soybean roots by three species of root-knot nematodes was detected by Balasubramanian (1971). Plants inoculated with M. javanica suffered reduction of root nodulation and showed a higher root-knot index than plants receiving M. incognita or M. hapla. Root-hair production was less on plants inoculated with the nematodes. Inoculum levels of 100 and 1,000 juveniles resulted in marked nodule reduction whereas 10 juveniles did not influence nodule formation.

Inhibition of nodule development on soybean was found to be greatest when Heterodera glycines race 1 was added with Rhizobium japonicum simultaneously or shortly after adding R. japonicum (Barker et al., 1972). Introduction of H. glycines 14 days after R. japonicum resulted in only slight inhibition of nodulation. Although race 1 was observed to penetrate nodular tissue, unlike race 4, development of juveniles of either race was inhibited by nodular tissue. Induction of giant cell formation was repressed by nodule tissue in the few cases where juveniles had developed into mature cysts.

d. The Effect of Organosphosphate Nematicides and Synergists on Infectivity and Development of Root-knot Nematodes

Information concerning the use of systemic organophosphate insecticides for the control of various plant parasitic nematodes, including Meloidogyne spp. is well documented in the scientific literature.

Although Metcalf (1955) stated that absorption of systemic insecticides by plant roots is relatively inefficient, soil application of these pesticides has been widely used by other workers. Sasser et al., (1951), in preliminary tests, found that soil drenches of Systox resulted in control of root-knot nematodes on tomatoes but concentrations of Systox used were highly phytotoxic. These workers also found that although Heterodera rostochiensis juvenile emergence from cysts was inhibited, and hatched juveniles were killed at all concentrations of Systox used, soil drenches resulted in low germination of potato seed, poor foliage growth and no reduction of golden nematode cyst formation. Later, Sasser (1952) observed reduction of infection and reproduction of root-knot nematodes on tomatoes and cucumbers using Systox concentrations ranging from 0.005 to 1.0%. At concentrations greater than 0.1%, the chemical was phytotoxic to both plants. Sasser noted slight inhibition of egg hatch and paralysis of hatched juveniles in solutions of Systox. Reduction of Pratylenchus penetrans in roots of Easter Lillies, from 600 per g in control roots to 37 per g in treated roots, was achieved with soil application of Thimet, an organophosphate soil insecticide (Jensen and Konicek, 1960). Elimination of Meloidogyne hapla from tubers and roots of potatoes, with only slight phytotoxicity, was accomplished using seed piece dips in 800 ppm solutions of Dimethoate (Helton, 1964). Using pot experiments designed to simulate field conditions,

Gersdorf and Lucke (1965) concluded that the systemic nematicide, Disyston, did not prevent new infections of Meloidogyne sp. or reduce infections already present on potato. Helton (1965) showed that soils drenched with Dimethoate at 50 to 1,800 ppm were effective in reducing root-knot of tomatoes. Phytotoxicity was not observed below 1,800 ppm. Winchester and Averre (1966) compared several nematicides in attempts to control Meloidogyne sp. on tomato seedlings. They reported near perfect control with the systemic organophosphate Dasanit. Damage to gladiolus corms by Meloidogyne sp. was decreased by Parathion, Zinophos, Thimet and Sarotex applied over the corms in open furrow or in advance of the growing roots (Overman, 1967). Soil applications of Dasanit, Diazinon, Cynem, Phorate and Temik controlled sting and stubby-root nematodes for up to 12 weeks and promoted good growth of several vegetable crops (Brodie, 1968).

Effective control of root parasitic nematodes has also been achieved by some workers using foliage applications of systemic insecticides. Completely ineffective control of Heterodera rostochiensis on potatoes and Meloidogyne sp. on tomato and tobacco seedlings was observed by Sasser et al., (1951) using foliar sprays of Systox ranging from 0.006 to 1.0%. At the 1% concentration, burning and curling of the leaves of sprayed plants were noted by these workers. Populations of Meloidogyne hapla on Dimethoate sprayed potato plants were completely eliminated from the tubers and roots by foliage run-

off sprays of 800 ppm (Helton, 1964). A year later, Helton (1965) reported suppression of root-knot nematode development on tomato by foliar sprays of Dimethoate at 1,800 ppm. Symptoms of phytotoxicity were not observed below 1,800 ppm. Also effective in reducing root-knot damage in tomato is Vamidothion which was found by Dantas and da Matta (1971) to be more effective than Metasystox when applied to the foliage at 15 and 30 days after Meloidogyne incognita infection. These workers further observed that the number and weight of fruit was increased markedly by Vamidothion application.

Interesting work has been reported concerning the use of dimethyl sulfoxide (DMSO). Although research on DMSO has been primarily concerned with potential medical benefits (Leake, 1966) its solvent and membrane penetrant properties have led to its being tested as a synergist in plant disease control. Keil et al., (1965), noted that control of bacterial leaf spot of peaches was significantly enhanced by spraying low concentrations (28 to 42 ppm) of oxytetracycline with 0.25 to 2.0% DMSO in the spray mixture. Sprays containing DMSO alone were ineffective, as were Dodine and zinc sulfate, both alone and in combination with DMSO. Using radioactive DMSO, Keil et al., (1967) demonstrated greater radioactivity in peach leaves sprayed with a DMSO - oxytetracycline combination than DMSO alone, indicating that the antibiotic augmented leaf uptake of DMSO. Synergism between DMSO and sodium pentachlorophenate

mixtures was observed by Corbin et al., (1966) in the reduction of radial mycelial growth of Monilinia fructicola on an agar medium. No synergy was observed by Pine (1967) when testing the reactions of virus inoculated peach trees to various combinations of DMSO and known viral inhibitors. Erdman and Hsieh (1969) detected significant increase in uptake of calcium, magnesium, potassium and phosphorous with concentration of DMSO. However, because of phytotoxic effects at high concentration, these workers recommended that concentrations of less than 0.1% be used where DMSO is employed as a synergist for pesticides or fertilizers. Use of DMSO in research concerning control of plant root pathogens, such as nematodes, fungi or bacteria, has to this writer's knowledge, never been attempted.

PART I

STUDIES OF SAPROZOIC NEMATODES

1. Feeding and Multiplication of Saprozoic Nematodes on Rhizobium spp.

A well established phenomenon in nature is that the rhizosphere of plants contains greater numbers of microorganisms than the adjacent, non-rhizosphere soil (Krasilnikov, 1958; Lochead, 1959; Starkey, 1959). This applies to various microorganisms in soil, including nematodes (Katznelson, 1965). It has been noted by Henderson and Katznelson (1961) that free-living nematodes are found in greater numbers in the rhizosphere of several economic plants, than in non-rhizosphere soil. Jensen and colleagues (Jensen, 1967; Jensen and Gilmour, 1968; Jensen and Siemer, 1969; Chantanao and Jensen, 1969a,b; Jensen and Stevens, 1969) have studied the feeding ability of saprozoic nematodes on certain members of the soil microflora, such as bacterial cells, fungal spores, and bacterial viruses, in relation to the survival of these organisms. They have been concerned with the possible role of saprozoic nematodes in the epidemiology of soil-borne plant diseases, and consequently, their studies have focused mainly on plant-pathogenic microorganisms. No work has been done, however, on the growth of saprozoic nematodes on Rhizobium spp., the symbiotic bacterial

partners of legumes that initiate nodule formation. Several experiments were undertaken, therefore, to determine whether saprozoic nematodes were capable of ingesting and multiplying on different Rhizobium spp.

a. Methods and Materials

(i) Isolation and Culture of Rhizobium spp.

An attempt was made to isolate Rhizobium spp. from a commercial legume seed inoculant, Legume Aid[a]. A moistened sterile glass rod was dipped into the black, powdery inoculant and streaked lightly over the surface of Nutman's yeast extract - mannitol agar (Bergersen, 1961) contained in 100 mm Petri dishes. Five replicates were used. After streaking, the plates were incubated at 24°C for three days. Examination of the plates revealed a mixture of several different microorganisms. Large, rapidly spreading colonies of Rhizobium spp. were found along with a smaller number of yeast and fungal colonies. Due to the presence of contaminating organisms and the lack of control over selection of specific Rhizobium sp. from the general purpose inoculant, a method of obtaining pure cultures of Rhizobium sp. from legume nodules was used.

Large, mature nodules were removed from the root systems of the following legumes: bean (Phaseolus vulgaris L.), cultivar Slendergreen; red clover (Trifolium pratense L.), cultivar Dollard; and a common garden vetch (Vicia sp. L.). The nodules

[a]Agricultural Laboratories Inc., Columbus, Ohio

were washed vigorously several times in tap water to remove closely adhering particles of soil, placed in sterile, rubber stoppered 50 ml Erlenmeyer flasks and surface sterilized in a solution of 0.1% $HgCl_2$ for five minutes. The nodules were frequently agitated to expose all areas equally to the chemical. The $HgCl_2$ was then drained off and the nodules washed three times with sterile distilled water. One ml of sterile distilled water was then added to each flask and the nodules were crushed, using a sterile glass rod. This left a very dense population of bacterial cells and nodule remnants in each flask. In order to obtain pure cultures of each type of Rhizobium sp. (R. phaseoli, Dangeard, from Phaseolus vulgaris L.; R. trifolii, Dangeard, from Trifolium pratense L.; and R. leguminosarum, (Frank) Baldwin and Fred, from Vicia sp. L.) a series of dilution blanks were prepared. Eight 15 x 150 mm screw cap culture tubes containing 9 ml of distilled water were autoclaved and after cooling, one ml of the crushed nodule suspension was transferred to the first tube, using a sterile pipette. The contents were thoroughly mixed and one ml was transferred to a second tube using a sterile pipette. This procedure was repeated in succession giving a series of dilutions from 10 to 100,000,000. One ml aliquots were then transferred from each dilution to sterile 100 mm Petri dishes. Three replicates per dilution were used. Sterile Difco Nutrient Agar, cooled to 45°C, was then added to each plate and the contents agitated gently in a swirling

motion to allow the rhizobial cells to disperse evenly throughout the agar medium. The plates were incubated at 25°C for four days and examined for the presence of bacterial colonies. In general, platings of dilutions 10 to 100,000 were so heavily populated with colonies that enumeration and morphology studies were impossible. The most successful dilutions were in the range of 1,000,000 to 10,000,000. Colony morphology and colour appeared uniform in all plates. Colonies were quite small and did not appear to increase in size after four days. Because of the inadequate nutrition provided by Difco Nutrient Agar, three different recommended media were tried including congo red mannitol agar (Harrigan and McCance, 1966), Nutman's yeast extract - mannitol agar and Norris' synthetic medium (Bergersen, 1961). Samples of Rhizobium sp. from bean, clover and vetch isolates were streaked over the surface of the above media and growth compared after incubating at 25°C for four days. Of the three media, congo red mannitol agar (CRMA) supported the most abundant growth. Colonies appeared opaque, white and mucoid on this medium. The pink colour of CRMA provided a good contrast for the spreading, white growth of Rhizobium. The composition of the medium is as follows: mannitol, 10g/l; K_2HPO_4, 500mg/l; $MgSO_4 \cdot 7H_2O$, 200mg/l; NaCl, 100mg/l; $CaCO_3$, 3.0 g/l; yeast extract, 1.0g/l; agar, 15.0 g/l; congo red, 1% aqueous solution, 2.5ml/l; distilled water, 1,000 ml.

Samples of the bean, clover and vetch isolates were transferred to slants of CRMA for study of growth characteristics

of the different species or strains of Rhizobium. Observations of growth from streak inoculations and phase-contrast microscopic studies at 450 and 1,000 magnifications indicated that the isolates were representative of R. phaseoli, R. trifolii and R. leguminosarum respectively (Breed, Murray and Smith, 1957).

Cultures were maintained at 4°C on CRMA slants and transferred to fresh media at monthly intervals.

(ii) Isolation and Culture of Saprozoic Nematodes

Cultures of unidentified species of Chiloplacus (Thorne) and Cephalobus (Bastian) were obtained from Dr. R. H. Estey, Department of Plant Pathology, McGill University, Macdonald Campus. Two isolates (R_{1a} and R_2) of Rhabditis (Dujardin) were extracted from a soil sample taken from the root zone of Slender-green beans grown in the Department of Plant Pathology Experimental field, at Ste. Anne de Bellevue, P. Quebec. The Baermann funnel technique of extracting nematodes from soil was used (Cairns, 1960). The nematodes were maintained on a mixture of unknown soil bacteria cultured on a modified Difco Nutrient Agar (0.8 g/l Nutrient Broth powder; 15.0 g/l agar) medium. The bacteria multiplied extremely rapidly on the full strength Difco Nutrient Agar (8.0 g/l) and in several instances, appeared to retard the multiplication of the nematode in question. This may have been due to the decreasing oxygen tension of the medium caused by the rapidly dividing bacteria, and the high requirements of this nematode for oxygen (Nicholas and Jantunen,

1966). Also, due to rapid multiplication of the nematodes and subsequent loss of water from the Petri dish, the agar began to crack and shrivel. In order to overcome these problems the nematodes were subcultured to fresh modified Difco Nutrient Agar every month, and the dishes were placed in polyethylene bags to conserve moisture. It should be pointed out that the nematodes were reared in this way only for routine population maintenance and for experiments where very small numbers of nematodes were required. For different methods of rearing very large populations of saprozoic nematodes, see Part I, section 4.

Unless otherwise mentioned, individual nematodes were handled and transferred using Kerr^{R} Root Canal Files, Style D, Number 1.

(iii) Surface Sterilization of Nematode Eggs

Saprozoic nematodes transferred to the modified Difco Nutrient Agar carried bacteria with them. Twenty-four hours after transfer, the bacteria had multiplied to such an extent that colony formation was visible. At this point the nematodes had begun to feed vigorously on the edge of the bacterial mass, and, in the subsequent process of migration over the plate, bacteria were transferred to fresh uncolonized portions of the nutrient agar surface. Egg laying began three days after transfer, although a few mature gravid females deposited eggs after only one day. As soon as a colony of bacteria was located by the actively moving nematodes, the movements across

the plate ceased and feeding began to take place. Often only the head parts of the nematodes could be seen to move, the rest of the body remaining quiescent. Eggs were laid while the nematodes fed, often totalling as many as 10 to 20 per female. The eggs were found usually in a group, giving the plate a "patchy" appearance, each patch consisting of clusters of eggs. The concentration of the agar used in the medium influenced the capacity of the nematodes to migrate through it. When the concentration of agar was less than 1.5% (15.0 g/l), the nematodes easily moved downward into the medium, and in many cases, often deposited their eggs under the surface. An agar concentration of 2% (20.0 g/l) provided sufficient firmness to the medium to prevent the nematodes from moving below the surface; therefore, all eggs deposited were left on top of the medium. This facilitated direct access to the eggs and enabled collections to be made easily and rapidly.

It was found that the state of embryonic development within the egg, at the time of egg collection, was extremely important for surface sterilization treatments. Embryonic development, was, therefore, followed closely. Immediately following egg deposition by gravid females, initiation of embryo development occurred, with the blastula and gastrula stages forming after three to five days. Eggs were collected shortly after gastrulation took place. At this time the embryo consisted of a small number of angular cells (ectoderm)

with one end of the embryo appearing darker than the other. Eggs which were collected at this time easily withstood the surface sterilization process, whereas those collected prior to gastrulation seldom hatched after surface sterilization treatments.

After collection, 24 to 48 hours were required before recognition of the vermiform shape within the egg was possible. Hatching followed quickly, usually within an additional 24 hours.

With the aid of a Wild M-5 stereomicroscope (from Wild of Canada, St. Lambert, Quebec) equipped with a base for transmitted light and handrests, eggs were collected with non-sterile Pasteur pipettes drawn from 6 mm glass tubing and plugged at one end with cotton. Rapid collection of mature eggs was made possible by drawing out the narrow end of the pipette to 1.0 mm. Moistening of the pipettes prior to egg collection prevented sticking of the eggs to the inner glass wall of the pipette. Also, the addition of a few drops of water over the area of agar containing the eggs made it easier to dislodge eggs from the viscous bacterial surface.

After the eggs were collected, they were transferred to a depression slide (75 x 25 mm, with a concave circular depression, 16 mm diameter x 3 mm depth) containing a few drops of 0.75% NaCl. The saline solution prevented osmotic bursting of the eggs which occurred when the eggs were placed in distilled or tap water. The eggs were washed three times lightly to remove excess bacteria and particles of agar drawn into the

pipette along with the eggs. Then, using a sterile Pasteur pipette, the eggs were covered with a 0.05% solution of NaOCl (commercial bleach, Javex) and left for 15 minutes. After this time the NaOCl was drawn off and the eggs rinsed three times with sterile 0.75% NaCl. All operations were carried out on the stage of the stereomicroscope mentioned above. Several tests were made to check the surface sterilizing efficiency of the NaOCl treatment. For example, treated eggs, and untreated eggs for control, were transferred to sterile plates of modified Difco Nutrient Agar. No bacteria developed on the plates of treated eggs before or after hatching, whereas the control plates were covered with bacterial colonies. A test was also made using sterile Nutrient Broth. Treated and untreated eggs were added to the broth and after 24 hours at 24°C, it was examined for turbidity. The broth containing the treated eggs remained clear, whereas a cloudy, turbid solution formed within 24 hours in the broth to which untreated eggs were added.

(iv) Transfer of Nematode Eggs to Rhizobium spp.

In order to clearly observe egg embryonic development, the process of hatching, and feeding habits of the emerged juveniles on colonies of Rhizobium spp., it was necessary to use an agar medium which transmitted sufficient light to reveal the internal features of the maturing embryos and young juveniles. The previously used CRMA, although excellent for the growth of

Rhizobium, did not allow sufficient transmitted light for easy visualization of nematodes on the surface. Since calcium carbonate was responsible for the agar's opacity, a modified CRMA was devised. The composition of this medium is as follows: mannitol, 10 g/l; K_2HPO_4, 500 mg/l; $MgSO_4 \cdot 7H_2O$, 200 mg/l; NaCl, 100 mg/l; $CaCl_2$, 200 mg/l; yeast extract, 1.0 g/l; agar, 20 g/l; distilled water, 1,000 ml. A second problem was then encountered with the medium. Growth of Rhizobium was so great that nematodes feeding within the bacterial mass could not be seen due to the opaqueness of the bacterial cells. A slower, more controlled growth was obtained using the following concentrations: mannitol, 500 mg/l; K_2HPO_4, 500 mg/l; $MgSO_4 \cdot 7H_2O$, 200 mg/l; NaCl, 100 mg/l; $CaCl_2$, 200 mg/l; yeast extract, 50 mg/l; agar, 20 g/l; distilled water, 1,000 ml. The above medium was prepared and poured into 60 mm diameter sterile Petri dishes. After solidification the plates were streaked with the desired Rhizobium sp. and incubated at 24°C for 24 hours. Eggs of Rhabditis sp. were collected, surface sterilized according to the above method and deposited, onto and around, the small bacterial mass growing from the streaked area. The plates were placed in polyethylene bags, to prevent drying of the medium, and incubated in the dark at 24°C. Hatching and feeding movements of the juveniles were observed daily under the stereomicroscope at 20 to 100 magnifications.

b. Results

(i) Experiment One

In this experiment, the feeding habits and growth, to the adult stage, of three saprozoic nematodes on three species of Rhizobium was followed, using modified Difco Nutrient Agar. Nematodes used were two isolates of Rhabditis, both unnamed at the species level, but sufficiently different morphologically to be easily identified, and a Chiloplacus sp. The Rhizobium species were R. phaseoli, R. trifolii and R. leguminosarum. Preparation, collection and chemical treatment of nematode eggs followed the procedures outlined previously. Rhizobium spp. were streaked onto modified Difco Nutrient Agar and incubated for two days before addition of approximately 50 surface sterilized eggs to these and to control plates. The cultures were incubated at 24°C in the dark, with nematode growth and activity noted at 1 to 2 day intervals, for a period of 14 days. The results are summarized below in Table 1.

It is apparent from Table 1, that the nutrient requirements necessary for growth of saprozoic nematodes are provided by living cells of Rhizobium spp. grown on modified Nutrient Agar (Difco). Although measurements of nematode size were not taken, visual estimates of development indicate that, over a time period of 14 days, increase in size occurred slowly from juvenile to adult stage. Control experiments (without Rhizobia) failed to support nematode growth.

Table 1. Development[a] of two isolates of Rhabditis and one of Chiloplacus on three species of Rhizobium cultured on modified Difco Nutrient Agar

Rhizobium spp.	Rhabditis sp. Isolate R_{1a} Time (days)						Rhabditis sp. Isolate R_2 Time (days)						Chiloplacus sp. Time (days)				
	2	3	4	6	8	14	2	3	4	6	8	14	1	3	5	9	11
R. phaseoli	+	+	+	++	++	++++[b]	+	+	+	+	+	++	+	++	++	+++	++++[b]
R. trifolii	+	+	+	++	++	++++[b]	+	+	+	++	++	+++	-[d]	-	-	-	-
R. leguminosarum	+	+	+	++	+++	++++[b]	+	+	++	++	+++	++++[b]	-[d]	-	-	-	-
Control (No Rhizobium)	+	+	+	+	+	+[c]	+	+	+	+	+	+[c]	+	+	+	+	+[c]

[a] + = very small, ++ = small, +++ = medium, ++++ = large (adult)

[b] Although adult stage had been reached, egg deposition did not occur.

[c] Juveniles were alive and active after 11 and 14 days.

[d] Data not taken for these bacteria due to insufficient number of Chiloplacus eggs.

There appeared to be little difference in growth rate between nematode species on the different Rhizobium isolates. Chiloplacus sp., although a much larger nematode than the two isolates of Rhabditis, demonstrated a similar development rate. Isolate R_2 of Rhabditis sp. appeared to grow more slowly on R. phaseoli than on R. trifolii or R. leguminosarum. This may have been due to a smaller amount of bacterial inoculum of R. phaseoli than the other species.

Periodic phase contrast microscopic examination of the Rhizobium spp. during growth of the nematodes revealed the presence of a mixture of normal rod-shaped Rhizobium cells and much larger Y, star and oval bacteroid forms. The bacteroid forms, which predominated the total bacterial growth, have been found to be affected by the kind of medium on which the Rhizobium species are grown (Jordan and Coulter, 1965). It can be assumed, therefore, that the nematodes were able to feed and grow on these bacteroid cells of Rhizobium.

(ii) Experiment Two

The objective of this experiment was to determine whether Rhizobium spp. would support the growth and development of the R_2 isolate of Rhabditis sp. to the egg laying stage. Rhizobium spp., the same as in Experiment One, were streaked onto plates of modified CRMA and 10 to 20 surface sterilized eggs were transferred to each plate. All cultures were incubated at 24°C. The plates were examined every three days for nematode development and presence of eggs. The results are summarized in Table 2.

The data presented in Table 2 indicate that different species of Rhizobium, grown on modified CRMA, support nematode growth and deposition of eggs by the Rhabditis sp. (isolate R_2).

Initially, a few eggs were deposited at 12 days. This was followed by rapid egg production which continued for several days. It should be pointed out that due to the variation in maturation rate of individual nematodes, egg formation took place over an extended period of time, although it was possible to detect an initial "burst" in egg formation and deposition. Different Rhizobium spp. did not influence the time of egg deposition.

As soon as egg hatch occurred, the juveniles moved quickly to the bacterial mass and began feeding on the periphery of the colony. Feeding in this position continued for several days until the nematodes had grown to the small size stage (++). The larger juveniles then moved into the bacterial mass, remaining there for several days at a time. This was most likely due to the drying out of the agar medium surrounding the moist bacterial growth.

Following egg hatch in the control treatment, juveniles moved randomly over the plate, and, although not growing in size, they continued to be active until the experiment was terminated at the twenty-first day. Although several juveniles died before 21 days elapsed, the majority were actively moving but at a much slower rate than when the experiment was initiated.

Table 2. Effect of *Rhizobium* spp. on nematode growth and egg production by the R_2 isolate of *Rhabditis* sp.

	R. phaseoli		*R. trifolii*		*R. leguminosarum*		Control	
Time (days)	Nematode Growth[a]	Egg Deposition	Nematode Growth[a]	Egg Deposition	Nematode Growth[a]	Egg Deposition	Nematode Growth[a]	Egg Deposition
3	+	-	+	-	+	-	+	-
6	++	-	++	-	++	-	+	-
9	++to+++	-	++to+++	-	++to+++	-	+	-
12	++++	+[b]	+++	+[b]	+++	+[b]	+	-
15	++++	+[c]	++++	+[c]	++++	+[c]	+	-
18	++++	+	++++	+	++++	+	+	-
21	++++	+	++++	+	++++	+	+[d]	-

[a] + = very small, ++ = small, +++ = medium, ++++ = large (adult)

[b] After 12 days, few eggs were deposited.

[c] Egg deposition now rapid.

[d] Juveniles alive and active after 21 days.

2. Feeding and Multiplication of Saprozoic Nematodes on Soil Yeasts

Despite the enormous effort in the exploitation of yeasts for industrial purposes, and the concomitant increase in knowledge, very little is known about the role of yeasts in the ecology of microorganisms of the soil. Among the few studies that have been completed, techniques for isolation of yeasts from soil have been developed (di Menna, 1957; Miller and Webb, 1954; Carmo Sousa, 1969) and yeasts from certain plant rhizospheres have been identified (Babeva and Saveleva, 1963; Babeva and Belyanin, 1966; Last and Price, 1969). Since it is known that: (1) yeast cells are often as large as fungal spores, (2) saprozoic nematodes such as Rhabditis sp. are found in plant rhizospheres and (3) saprozoic nematodes can ingest fungal spores (Jensen, 1967; Jensen and Siemer, 1969), experiments were designed to determine whether saprozoic nematodes could grow and develop on soil yeasts and whether these members of the soil rhizosphere microflora would support nematode egg formation. The only experiment found in the literature was an investigation by McCoy (1929) who used several bacteria and a single yeast, Torula rosea, in determining whether larvae of Ancylostomum caninum, an animal parasitic nematode, would be adequately supplied nutritionally. The experiment was negative with the yeast.

a. Methods and Materials

(i) Culture of Yeasts

The following yeasts were obtained from the Department of Microbiology, Macdonald College, Ste. Anne de Bellevue, Quebec: Candida mycoderma, Lodder and Kreger-van Rij; Hansenula schneggi, (Weber) Wickerham; Rhodotorula pallida, Lodder; Saccharomyces cerevisiae, Hansen; and Torulopsis magnoliae, Lodder and Kreger-van Rij. Cultures were maintained at 4°C on slants of Difco Malt Agar, with transfer being made to fresh media every month.

In order to observe the feeding habits and development of saprozoic nematodes on the different yeasts, a form of Wickerham's medium was used (Wickerham, 1951). The medium contained glucose, 10 g/l; malt extract, 3 g/l; yeast extract, 3 g/l; peptone, 5 g/l; agar, 20 g/l; and distilled water, 1,000 ml.

(ii) Saprozoic Nematodes

The R_2 isolate of Rhabditis sp. was used throughout the studies with yeasts. Methods of egg collection, surface sterilization, transfer to yeast cultures, incubation and stereomicroscopic observation were the same as those used in the previous studies involving bacteria.

b. Results

(i) Experiment One

This experiment dealt with the growth and development of Rhabditis sp. (isolate R_2) on several species of yeasts.

Approximately four to five eggs were placed near each colony of yeast, using aseptic technique. Observations were made at three day intervals over a twelve day period. The experiment was terminated after twelve days due to the excessively dense growth of the yeasts which made observation of nematode size and movement difficult. The results are summarized in Table 3.

The data indicate that isolate R_2 of Rhabditis sp. is capable of consuming living cells of five different yeasts and of developing rapidly to the medium-large stage, prior to becoming adult size. Rhodotorula pallida appeared to support the most rapid growth of the nematode in the early stages of development. Growth and development on Saccharomyces cerevisiae was slower than on other yeasts. This was due mainly to the difficulty with which the juveniles fed on the large cells of this yeast. Egg deposition was not observed.

(ii) Experiment Two

Following the above demonstrated evidence that a saprozoic nematode can utilize yeast cells and develop through the first three juvenile stages (Table 3), it was decided to determine whether such development would continue to the adult egg-laying stage.

In order to observe clearly the growth and development of the nematodes in the yeast colonies, a further modification of the above medium was employed. The following concentrations

Table 3. Development[a] of *Rhabditis* sp. (isolate R_2) on five yeasts cultured on modified Wickerham's Agar

	Time in Days			
Yeast	3	6	9	12
Candida mycoderma	+	+	++	+++
Hansenula schneggi	+	+	++	+++
Rhodotorula pallida	+	++	+++	+++
Saccharomyces cerevisiae	+	+	++	++
Torulopsis magnoliae	+	+	+++	+++
Control (No Yeast)	+	+	+	+[b]

[a] + = very small, ++ = small, +++ = medium, ++++ = large (adult)

[b] Juveniles were alive and active after 12 days.

were used: glucose, 250 mg/l; agar, 20 g/l and distilled water, 1,000 ml. This much-diluted modification of the standard medium devised by Wickerham, was found ideal for allowing a slower, controlled growth of the yeast isolates. Excellent light transmission, enabling rapid location of feeding nematodes in the yeast colonies, was also obtained using this modified medium.

Experimental methods and procedures were the same as in the above experiment, with the exception that approximately twenty eggs were added to each colony of yeast. Six replicates were used. The experiment was completed after twenty-one days. The results are compiled in Table 4.

From the data shown in Table 4, it can be seen that all yeast species supported the development of a Rhabditis sp. (isolate R_2) to the egg laying stage. Generally, eggs were laid in small numbers after 15 days, with rapid production occurring on and after day 18.

Although the initial number was small, egg formation and deposition occurred earlier on colonies of Rhodotorula pallida than on any other yeast. Nematode growth and maturation was also more rapid on R. pallida.

All growth phenomena, including molting and egg formation and liberation, appeared to be retarded by Saccharomyces cerevisiae. It is most likely that the delay was caused by the greater length of time required for the juveniles to seek out the smaller, more easily ingested cells of S. cerevisiae.

Table 4. Effect of different yeasts on development and egg deposition of a Rhabditis sp. (isolate R_2)

Yeast	Nematode Development	Time in Days 3	6	9	12	15	18	21
Candida mycoderma	Nematode Growth[a]	+	++	+++	+++	++++	++++	++++
	Egg Deposition[b]	-	-	-	-	+	+	+
Hansenula schneggi	Nematode Growth[a]	+	+	++	+++	++++	++++	++++
	Egg Deposition[b]	-	-	-	-	+	+	+
Rhodotorula pallida	Nematode Growth[a]	+	++	+++	++++	++++	++++	++++
	Egg Deposition[b]	-	-	-	+	+	+	+
Saccharomyces cerevisiae	Nematode Growth[a]	+	+	++	++	+++	+++	++++
	Egg Deposition[b]	-	-	-	-	-	-	+
Torulopsis magnoliae	Nematode Growth[a]	+	+	++	+++	++++	++++	++++
	Egg Deposition[b]	-	-	-	-	+	+	+
Control (No yeast)	Nematode Growth[a]	+	+	+	+	+	+	+
	Egg Deposition[b]	-	-	-	-	-	-	-

[a] + = very small, ++ = small, +++ = medium, ++++ = large (adult)

[b] - = no egg production, + = eggs produced.

A comparison of Table 4 and Table 2 shows that Rhizobium spp. are better nutritive sources than certain soil yeasts, in supporting the growth of saprozoic nematodes.

3. Effect of Saprozoic Nematodes on the Transfer of Cells of R. phaseoli into the Root Zone of Beans

Although recent research on the biology of saprozoic nematodes has shown that a variety of microorganisms, including bacteria (Dougherty and Calhoun, 1948; Dougherty, 1960), fungal spores (Jensen, 1967; Jensen and Siemer, 1969), mycoplasma (Jensen and Stevens, 1969), actinomycetes (Jensen and Gilmore, 1968) and algae (Nielsen, 1949b) are used as food substrates, it is generally agreed that bacteria comprise the major component of their diet (McCoy, 1929; Nielsen, 1949b Nicholas and Jantunen, 1966; and Sohlenius, 1968).

Because of their bacterial food preferences and involuntary movement in the medium in which they are suspended, saprozoic nematodes are capable of conveying bacterial cells short distances. This may be easily observed in the laboratory. When feeding on colonies of bacteria cultured on nutrient agar, the nematodes coat their bodies with bacterial cells, and in subsequent migratory movements to uncolonized areas on the medium, these cells are deposited at various points where they can be recognized by the appearance of colonies. In this way, the bacteria are moved from one substrate site to another.

In considering the ecology of soil microorganisms in general, and in particular, the distribution of Rhizobium, the question arises of the role of bacterial feeding nematodes in the transport of Rhizobium from one microhabitat to another. Can saprozoic nematodes, either through ingestion and passage through the digestive system, or by contamination of the outer cuticle, convey viable cells of Rhizobium phaseoli into the root zone (rhizosphere) of beans from a non-rhizosphere area? A number of experiments, based on the presence or absence of nodules on bean roots, were devised to determine whether nematodes of Rhabditis sp. (isolate R_2), Chiloplacus sp. or Cehpalobus sp., could transfer R. phaseoli in the above manner.

a. Experiment One

The purpose of this preliminary experiment was to determine the migratory ability of Rhabditis sp. through a cylindrical column of soil.

(i) Methods and Materials

Rhabditis sp. (isolate R_2) was reared on a mixture of soil bacteria grown on modified Difco Nutrient Agar. After several days a large population of nematodes had developed. The agar was then cut into small sections and transferred to Baermann funnels for nematode extraction. After 24 hours the nematodes were drawn off and washed several times in sterile water using a small laboratory centrifuge. This was done to remove the

large population of bacteria in the extracting fluid. After washing, the nematodes were concentrated in two ml of sterile distilled water.

The soil used in this experiment was an air dried sandy loam passed through a sieve of United States Standard (USS) number 10 mesh. The soil was transferred to five glass tubes (2 cm diameter x 7.5 cm length) and filled to a depth of 5 cm. The tubes were then stoppered with absorbent cotton, covered with aluminum foil and autoclaved in a vertical position at 101.3kPa (15 lb/in^2) for 15 minutes. The moisture holding capacity (m.h.c.) of the soil was determined to be 58%. Sterile water was added, after the soil in the tubes had cooled, to give the soil a moisture content of 60% of its m.h.c. The tubes were left for 2 hours to allow for moisture equilibration.

One drop of the previously prepared Rhabditis sp. suspension, containing 100 to 200 nematodes per drop, was then added to the surface of the soil in each tube. The inoculated soil columns were incubated at room temperature for periods of 12, 24, 36, 48, and 60 hours. At each time period, one tube was selected at random and, using a clean spatula, the soil column removed carefully in 12.5 mm portions. Each portion was transferred to a Baermann funnel and the number of nematodes determined using a Wild M-5 stereomicroscope. Counting was facilitated by use of a plastic disposable Petri dish (12 x 50 mm), with a grid etched into the underside of the dish. In

view of the preliminary nature of this experiment the data were not subjected to statistical analysis.

(ii) Results

A summarization of the results of the above experiment is found in Table 5.

Although the number of nematodes deposited on each soil column varied greatly, it is nevertheless evident from the data in Table 5, that Rhabditis sp. (isolate R_2) is capable of moving through a 5 cm column of soil. The depth of the soil had a profound effect on the numbers of nematodes migrating into the deeper parts of the column. Very few nematodes were isolated in the bottom portion. It is clear that the nematodes preferred to remain near the top of the soil tube. This was probably due to more favorable oxygen conditions, as it is known that Rhabditis sp. have an unusually high oxygen requirement (Nicholas and Jantunen, 1966). Longer incubation periods did not alter the above noted migratory pattern.

Table 5. Migration of Rhabditis sp. (isolate R_2) in a sandy loam soil

Section of Soil Column Sampled	Number of Nematodes Per Extracted Sample Time in Hours				
	12	24	36	48	60
Top Portion (Inoculated)	105	117	106	66	56
Second Portion	51	18	11	39	30
Third Portion	42	44	3	11	43
Bottom Portion	30	16	6	8	15

b. Experiment Two

In view of the results from experiment one, in which it was shown that a saprozoic nematode could migrate through a 5 cm soil zone, an experiment was conducted to determine whether cells of Rhizobium phaseoli could be transported through the soil into the rhizosphere of beans.

(i) Methods and Materials

The nematode used for this experiment was a Chiloplacus sp. Large populations were reared on a pure culture of Escherichia coli (Migula) Castellani and Chalmers cultured on modified Difco Nutrient Agar. The method by which the Chiloplacus sp. was cultured on R. phaseoli will be described later in this experiment.

The culture method for R. phaseoli was the same as that used in the feeding and multiplication experiments in section one.

Phaseolus vulgaris seed preparation (cultivar, Pencil Pod) consisted of soaking the seeds in tap water for 6 hours, surface sterilization in 0.1% $HgCl_2$ for 5 minutes and three rinses in sterile water. Seeds were then transferred to Petri plates of CRMA for germination. Examination was made for contaminating microorganisms. Upon emergence of the radicle, the germinated seeds were placed in a refrigerator ($5^{o}C$) to slow down growth, until required in the experiment.

In order to provide a sterile environment for bean root growth a special apparatus was used (Figure 1). The apparatus consisted of a 12cm wide-mouth glass bottle enclosed by a thick

layer of cotton covered on both sides by fibreglass screening and held together by staples. The cover, firmly attached to the bottle by a strong elastic band, contained two circular openings, each large enough to permit the fitting of two cotton-plugged glass tubes (18 mm diameter x 60 mm length). One tube served for the addition of inoculum (inoculation tube), the other for containing the rapidly growing bean seedling (planting tube).

Approximately 620 grams of a sandy loam soil were weighed and transferred to each bottle. The m.h.c. was determined to be 68%. The soil moisture content was adjusted to 60% of the m.h.c. by addition of distilled water. Each container was then assembled, the cover with fitted inoculation and planting tubes firmly attached, and the entire unit left 2 hours for soil moisutre equilibration. Vermiculite was added to the planting tube to a depth of approximately 2 cm and moistened lightly. The assembled units were then autoclaved for 2 hours at 101.3 kPa. When cool the pH of the autoclaved soil was determined to be 6.2, using a Beckman pH meter.

The units were then placed in an ultraviolet transfer chamber. Freshly germinated, microbial - free bean seeds, with radicle lengths of 1 to 2 cm were then inserted into the planting tube with the radicle immersed in the vermiculite layer. A small piece of water soaked, sterilized cotton was then placed immediately over the upper protruding seed surface in order to prevent rapid drying of the seed coat and subsequent abortive expansion of the cotyledons. The units were kept in

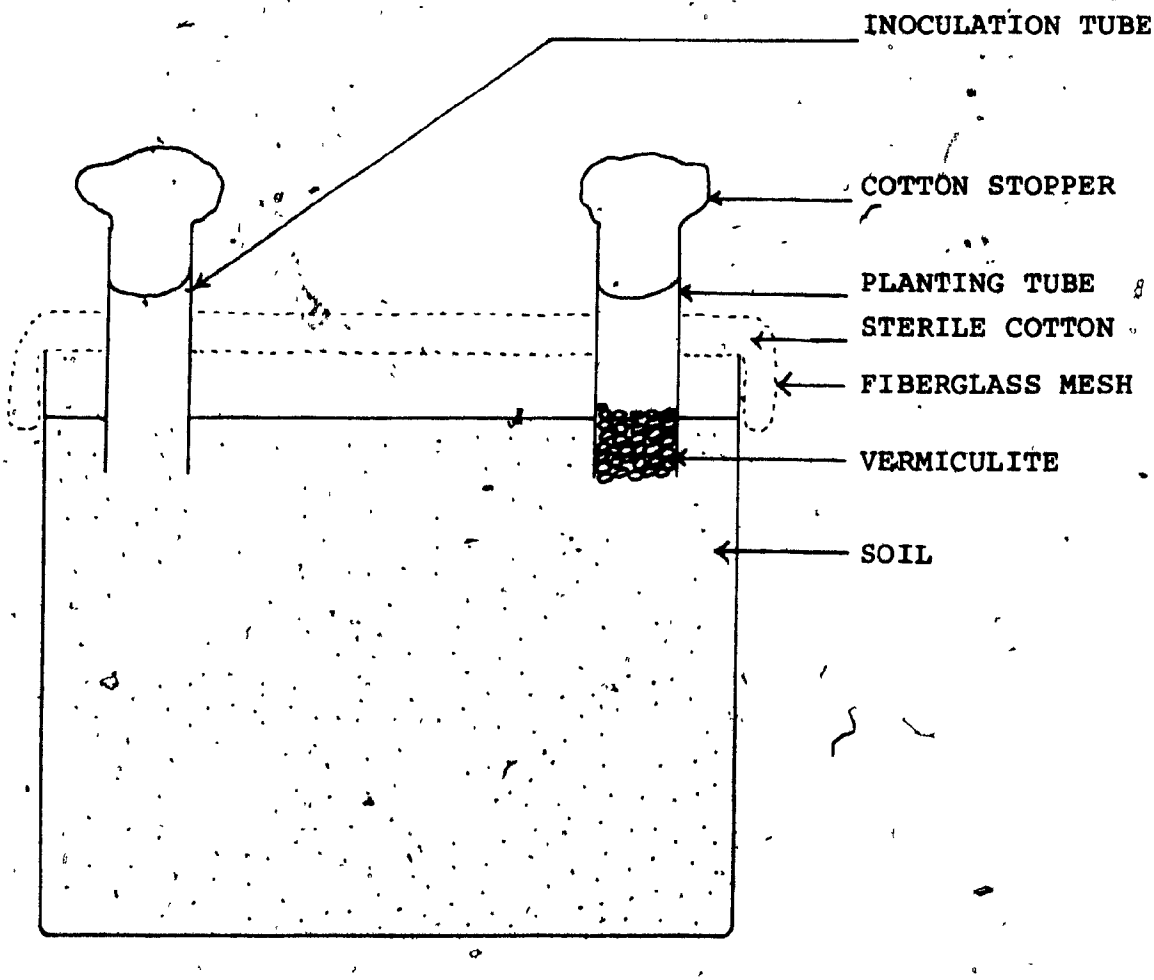


Figure 1. Diagram of apparatus employing sterilized soil for bean root growth.

the laboratory until all seedlings had emerged through the planting tube. As each seedling contacted and pushed off the cotton plug at the top of the planting tube, a fresh piece of sterile cotton was fitted evenly and firmly around the elongating hypocotyl. This was done to prevent microbial contamination of the root system. Upon emergence of the terminal leaflets from the planting tube, all units were wrapped with aluminum foil to keep light from the roots, and placed in a greenhouse. At various times during the experiment soil moisture determinations were made by removing the aluminum foil and visually examining the roots and soil. When required, sterile water was added through the planting tube, never through the inoculation tube.

The treatments in this experiment were as follows:

1. Uninoculated control
2. Chiloplacus sp. cultured on soil bacteria
3. R. phaseoli
4. Chiloplacus sp. cultured on R. phaseoli

In treatment two, approximately 1,000 Chiloplacus sp. were added to each inoculation tube, in 0.5 ml of sterile water.

In treatment three, 0.5 ml of an R. phaseoli suspension (5 loops from an R. phaseoli colony in 1.0 ml of sterile water in a small culture tube) was added to the inoculation tube, using a 1 ml pipette.

In treatment four, approximately 1,000 Chiloplacus sp., previously cultured for three days on CRMA plates of R. phaseoli and centrifuge-washed five times in sterile water, were added

to each inoculation tube in 0.5 ml of sterile water.

Upon completion of the treatments, each of which was replicated six times, all units were arranged randomly on a greenhouse bench. The temperature of the soil within each unit varied from 18 to 24°C. In addition to normal daylight, the plants received approximately 13,000 to 16,000 lx (1,200 to 1,500 foot candles) of artificial illumination for sixteen hours per day. The plants were allowed to grow for 24 days, after which they were carefully removed from the glass jars and the roots washed. Nodule counts were then made to determine whether a saprozoic nematode could transport cells of R. phaseoli from a non-root zone of soil into the rhizosphere of beans.

The data of this experiment, and, where appropriate, in subsequent experiments as well, were analyzed by the analysis of variance according to a randomized complete block design (Snedecor and Cochran, 1967). In those cases where such analyses indicated a significant treatment effect to exist, Scheffe's Test (Snedecor and Cochran, 1967) was employed to discriminate between individual treatment differences.

(ii) Results

The results of this experiment are summarized in Table 6.

Table 6. The effect of Chiloplacus on the movement of R. phaseoli into the rhizosphere of beans

Treatment	Number of Nodules per Plant Replicate 1	2	3	4	5	6	Total	Average
1. Uninoculated control	0	0	0	0	0	0	0	0^{a}[1]
2. Chiloplacus sp. cultured on soil bacteria	0	0	0	0	0	0	0	0^{a}
3. R. phaseoli	3	4	0	8	0	0	15	2.50^{a}
4. Chiloplacus sp. cultured on R. phaseoli	13	2	0	0	0	13	28	4.66^{a}

[1] Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

In preliminary tests, using autoclaved soil in 10 cm clay pots, control plants frequently became nodulated. Despite the use of autoclaved water and careful watering procedures, the appearance of nodules on control plants persisted. From the data in Table 6, it is clear that, using the apparatus shown in Figure 1, contamination by R. phaseoli was prevented.

The ability of Chiloplacus sp. to carry R. phaseoli cells through a soil medium and into the rhizosphere of Phaseolus vulgaris is supported by the data in Table 6. However, the results are somewhat confused by the appearance of nodules on plants in which only 0.5 ml of an R. phaseoli suspension was added to the inoculation tube. From this it can be inferred that either motile R. phaseoli cells moved along water films

on soil particles to plant roots, or that a portion of the bean root system grew very close to the inoculation tube. Although differences between treatment means were not significant (Appendix Table I), more nodules formed on plants treated with Rhizobium-fed Chiloplacus sp. than Rhizobium alone. This suggests that, under the conditions of this experiment, Chiloplacus sp. does play a role, perhaps minimal, in the movement of this biologically important bacterium through the soil.

c. Experiment Three

Because of the above apparent, but somewhat inconclusive evidence, that a saprozoic nematode can convey Rhizobium cells in soil to a legume host, it was decided to repeat the experiment using similar treatments but greatly modifying the apparatus, rooting medium and form of plant. It was hoped that by eliminating the periodic addition of water to the root system, the unwanted movement of R. phaseoli would be arrested. Therefore, the true role of saprozoic nematodes in relation to passage of bacterial cells in a particulate medium could be determined.

Accordingly, a technique of growing excised, nodule forming bean roots was used. Originally devised by Raggio and Raggio (1956) and later modified by Bunting and Horrocks (1964) and Cartwright (1967), this method provided an adequate nutrient-moisture environment for the successful growth and nodulation of isolated roots. A description of the above technique, plus necessary modifications, is outlined below.

(i) Methods and Materials

Excised roots were obtained from surface sterilized and germinated seeds of Phaseolus vulgaris cultivar, 'Pencil Pod' black wax bean. Upon emergence of the radicle the seedlings were transferred aseptically to culture tubes (150 x 18 mm) of sterile moist vermiculite closed with polypropylene Bacti - Capall's (from Fisher Scientific Co., Montreal). The moist vermiculite proved superior to soil or perlite in allowing straight, rapid, primary root elongation. The length of the rapidly growing root was easily determined by observing the growing radicle as it moved down the side of the tube. When the roots had reached a length of six to eight centimetres, the tubes were refrigerated until the roots were required for excising.

Dry silica sand was used as the rooting medium. One hundred and seventy grams of silica sand Number 24 (coarse grain) were placed in each of twenty-four 250 ml Erlenmeyer flasks. Onto this was layered 20 g of Number 8 silica sand (see Fig. 2). Each flask was wrapped with aluminum foil and oven sterilized for 4 hours at 175^{o}C.

When cool, an "inorganic" nutrient solution, similar to that used by Cartwright (1967), was added to the silica sand. The composition of the medium used was as follows: $CaCO_3$, 3.0 g/l; $MgSO_4 \cdot 7H_2O$, 700 mg/l; Na_2SO_4, 200 mg/l; KCl, 185 mg/l; KH_2PO_4, 200 mg/l; KI, 0.75 mg/l; $FeCl_3 \cdot 6H_2O$, 2.50 mg/l; H_3BO_3, 1.86 mg/l; $MnSO_4 \cdot 4H_2O$, 2.25 mg/l; $CuSO_4 \cdot 5\ H_2O$, 0.25 mg/l;

$ZnSO_4 \cdot 7H_2O$, 0.25 mg/l; $NaMoO_4$, 0.25 mg/l; and distilled water to 1,000 ml. The pH of the medium was determined to be 7.6. After preparation, 20 ml of this mineral-salt solution was pipetted to each vessel and left for three hours to allow for equilibration with the silica sand particles.

An inoculation tube (diameter 10 mm, length 20 mm) was gently inserted a small distance into the moist layer of No. 24 sand by carefully removing a small area of the coarse No. 8 layer. The tube was placed immediately adjacent to the wall of the flask. A rubber stopper, previously fitted with two cotton plugged glass tubes (diameter 4 mm, length 40 mm) and a glass vial (diameter 15, length 45 mm), was attached as shown in Figure 2. The intact units were then autoclaved for 30 minutes at 101.3kPa.

After autoclaving, the sterile units were transferred to an ultraviolet transfer chamber. The rubber stoppers were removed, inverted, and carefully placed onto the rim of the neck of the 250 ml Erlenmeyer flasks. Into each vial, now in an upright position, was poured a melted and cooled "organic" agar medium. Each vial was completely filled. Contained in this medium were the following chemicals: sucrose, 100 g/l; inositol, 1.0 g/l; nicotinic acid, 10 mg/l; pyridoxine, 2 mg/l; thiamine, 2 mg/l; agar 10 g/l and distilled water to 1,000 ml (Cartwright, 1967). Nicotinic acid required heating to 50°C in order to dissolve the crystals. When prepared, the organic

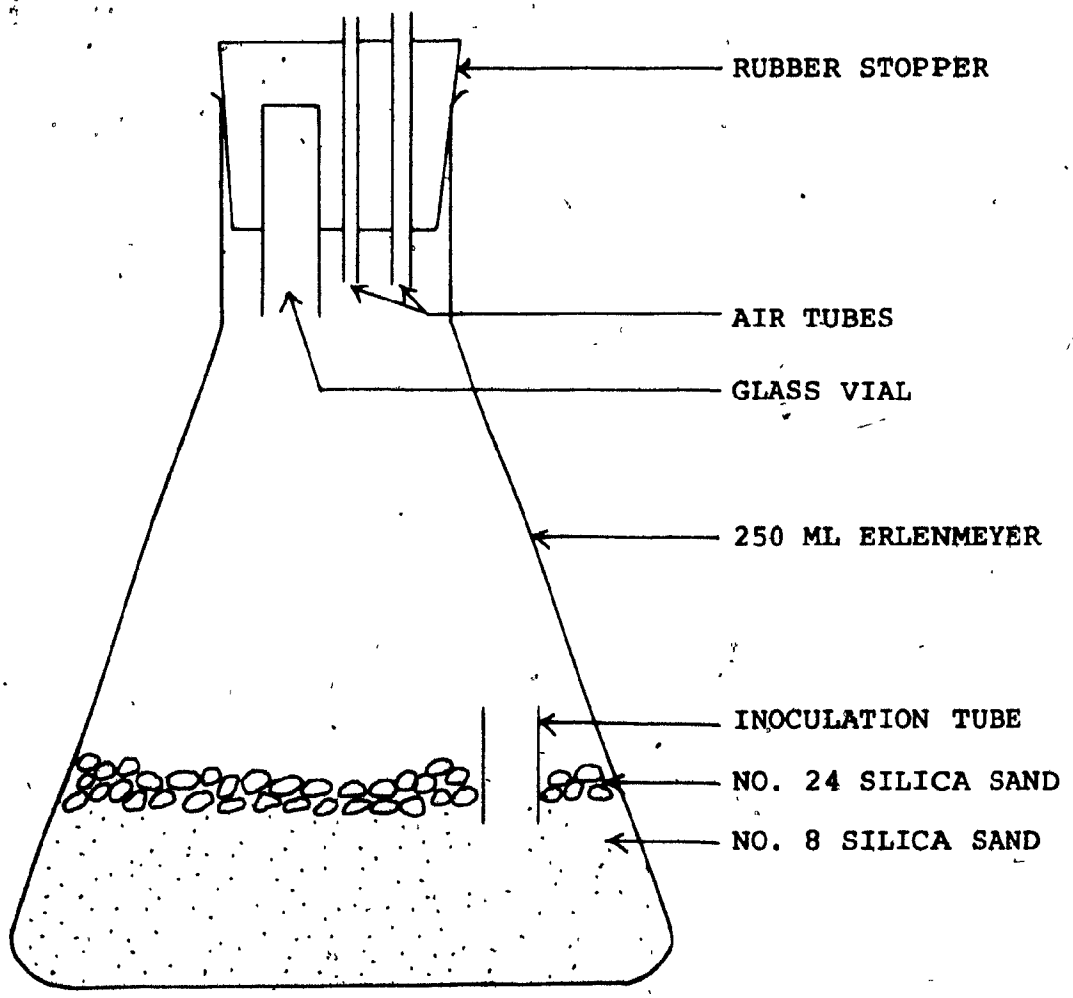


Figure 2. Diagram of apparatus for growing excised roots of beans.

agar medium was sterilized in cotton stoppered, 250 ml Erlenmeyer flasks, by autoclaving for 15 minutes at 101.3kPa.

As the melted agar in each vial was solidifying, the bean roots were excised. This was done in the sterile environment of the transfer chamber, using a sharp, sterile scalpel blade. The bean roots, prepared as above, were carefully removed from each tube, excess vermiculite gently brushed off, and the root plus a segment (approximately 10 mm) of hypocotyl tissue excised. Hypocotyl tissue was included as part of the explant because Bunting and Horrocks (1964) found that this tissue promoted greater nodule formation on excised roots of Phaseolus vulgaris. Using sterile forceps, the freshly cut end of each dissected root was inserted ten to fifteen millimetres into the solidified organic agar medium. The stopper was then replaced so that the root contacted the surface of the moistened silica sand medium. If the root was very long, and in danger of breaking as it struck the surface of the silica sand, a thin groove was scraped in the sand using a sterile glass rod. The root tip was then inserted into this groove.

The treatments were as follows:

1. Uninoculated control
2. Rhabditis sp. cultured on E. coli
3. R. phaseoli
4. Rhabditis sp. cultured on R. phaseoli

Treatment one consisted of excised roots and a drop of sterile water in each inoculation tube.

In treatment two, a drop of sterile water containing 600 Rhabditis sp. (isolate R_2) cultured on E. coli, was added to each inoculation tube. The details of nematode preparation were the same as those outlined above for Chiloplacus sp.

Treatment three consisted of a drop of R. phaseoli previously cultured on slants of CRMA. A suspension was made by adding five ml of sterile distilled water to a rubber stoppered agar slant culture tube and shaking violently several times so as to dislodge and evenly suspend the mucilaginous rhizobial growth.

Treatment four consisted of a drop of sterile water containing approximately 600 Rhabditis sp. (isolate R_2) cultured on R. phaseoli and prepared for inoculation as described previously for Chiloplacus sp.

In all treatments, the inoculum was placed directly into the small inoculation tube slightly embedded in the silica sand of each container.

Each treatment was replicated six times. All units were incubated at 24°C in a dark incubator for twenty-five days. After this time, the roots were removed carefully from the sandy medium, washed, and the number of nodules counted.

(ii) Results

In this experiment, under controlled conditions of moisture and temperature, the role of a saprozoic, bacterial feeding

nematode in transporting viable cells of Rhizobium was determined. The results are summarized in Table 7.

In comparison with the results of experiment two, in Table 6, the data in Table 7 are almost identical. Despite the more exacting conditions of moisture balance and temperature, cells of R. phaseoli, without the aid of nematodes, contacted and formed nodules on the growing excised roots. Although the motility of this bacterium may have been primarily responsible for the movement of the cells towards the excised roots, it is also possible that the cells remained in the immediate vicinity of the inoculation tube while the rapidly growing and branching roots moved near the inoculation tube, thereby facilitating contact between the two organisms.

Table 7. The effect of Rhabditis on the movement of R. phaseoli into the rhizosphere of excised bean roots

Treatment	Number of Nodules per Root — Replicate 1	2	3	4	5	6	Total	Average
1. Uninoculated control	0	0	0	0	0	0	0	0
2. Rhabditis sp. cultured on E. coli	0	0	0	0	0	0	0	0
3. R. phaseoli	0	1	2	0	23	0	26	4.3
4. Rhabditis sp. cultured on R. phaseoli	1	17	0	0	0	10	28	4.7

There was very little difference between roots treated with R. phaseoli and those treated with Rhizobium-fed Rhabditis sp., indicating that, under the conditions of this experiment, Rhabditis sp. was not effective in transmitting cells of R. phaseoli into the root zone of Phaseolus vulgaris. Because of this the data were not analyzed statistically.

Growth and development of the excised roots was rapid. Numerous, thin, white rootlets were produced immediately below the agar-immersed root segment. Many of these roots did not contact the silica sand below, and, in treatments where R. phaseoli was present, nodules were never formed on these aerial rootlets. Roots that contacted the silica sand were able to penetrate and utilize the nutrients available. This was especially so for the main root which was usually seen emerging through the sand at the bottom of the flask.

In all treatments (including the uninoculated controls), the inserted hypocotyl portion of the root frequently became necrotic toward the end of the experiment. Only rarely did this browning spread to the rootlets below. There was no apparent correlation between root browning and age of the roots. At the experiments termination, root tip browning was noticeable, but its occurance was variable between treatments.

Nodule formation appeared to affect the growth of the roots. In those treatments where nodules formed, root growth and development was more extensive than in non-nodulated

root systems of treatments one and two. It should be noted that, in comparison with the size of nodules formed on foliated bean plants, the majority of nodules formed on excised roots were small (1.0 to 2.0 mm in diameter). However, many of the larger nodules formed on excised roots were pink and had white bands around the periphery of the nodule which is characteristic of nodules formed by inoculated intact plants of Phaseolus vulgaris. Although nitrogen fixation was not determined, it may be assumed that this process occurred, since Raggio, Raggio and Burris (1959) demonstrated a positive nitrogen fixing ability for nodulated, excised bean roots. This may account for the above noted difference in root growth between nodulating and non-nodulating root systems.

d. Experiment Four

It was found in experiment two and three, that because of the natural motility of R. phaseoli and/or the movement of the bacterium on films of moisture over soil or silica particles, some roots of Rhizobium treatments were nodulating. This occurred despite the initial placement of the rhizobial inoculum some distance away from the growing root system. In each case, however, root growth took place in the direction of the inoculation tube. In order to avoid the occurrence of root nodulation in the above manner, the experiment was repeated using a different type of vessel and inoculation technique.

(i) Methods and Materials

Preparation of Pencil Pod bean seeds for germination, and later, for root excision, was the same as in the experiment outlined above.

The type of vessel used to grow the excised roots was a Blake culture bottle (capacity 1,000 ml: dimensions are 273 mm high and 63 mm x 95 mm at outside cross sections). Two hundred grams of dry silica sand, Number 24, was placed into each of thirty-two Blake culture vessels. The bottles were then placed flat and the silica evenly distributed. Fifty ml of Cartwrights "inorganic" medium was pipetted slowly into each bottle and allowed to equilibrate throughout the silica sand over a period of three hours. The bottles were then plugged with cotton and autoclaved at 101.3 kPa. for 30 minutes. Upon cooling, the autoclaved bottles were placed in a transfer chamber. This was immediately prior to addition of excised bean roots and various treatments. Autoclaved vials (diameter, 12 mm; length, 35 mm) were filled with warm "organic" agar medium and excised roots inserted after the agar solidified. Each vial and root explant was then carefully placed in a bottle of nutrient silica sand, with the root tip pointing in the opposite direction from the neck of the culture bottle (see Fig. 3). The vial of organic medium rested on the surface of the sand and the root tip gently contacted, but did not at this time penetrate, the moistened silica sand. Each culture bottle was

then fitted with an autoclaved rubber stopper containing a small cotton-filled air tube. Details of inoculum preparation for the different treatments will be described below.

The treatments used in this experiment are similar to those in the above experiment:

1. Uninoculated control
2. Cephalobus sp. cultured on E. coli
3. R. phaseoli
4. Cephalobus sp. cultured on R. phaseoli

Methods of culturing Cephalobus sp. on E. coli or R. phaseoli were the same as for Rhabditis sp. in experiment three. A significant change, however, was the use of small (10 mm) Number 2 Whatman filter paper discs as inoculation devices.

Treatment one consisted of excised roots and autoclaved inoculation discs inserted into the silica sand behind the vials of organic agar.

Treatment two consisted of excised roots and inoculation discs containing 1,000 Cephalobus sp. previously cultured on E. coli. The nematodes were cultured, collected in Baermann funnels, rinsed in sterile water and concentrated in a small volume of water. One drop, containing approximately 1,000 nematodes, was added to a folded sterile filter paper disc embedded in an autoclaved glass ring. Several drops of sterile distilled water were added to the nematodes in order to remove excess bacterial cells. The moist inoculum disc was then

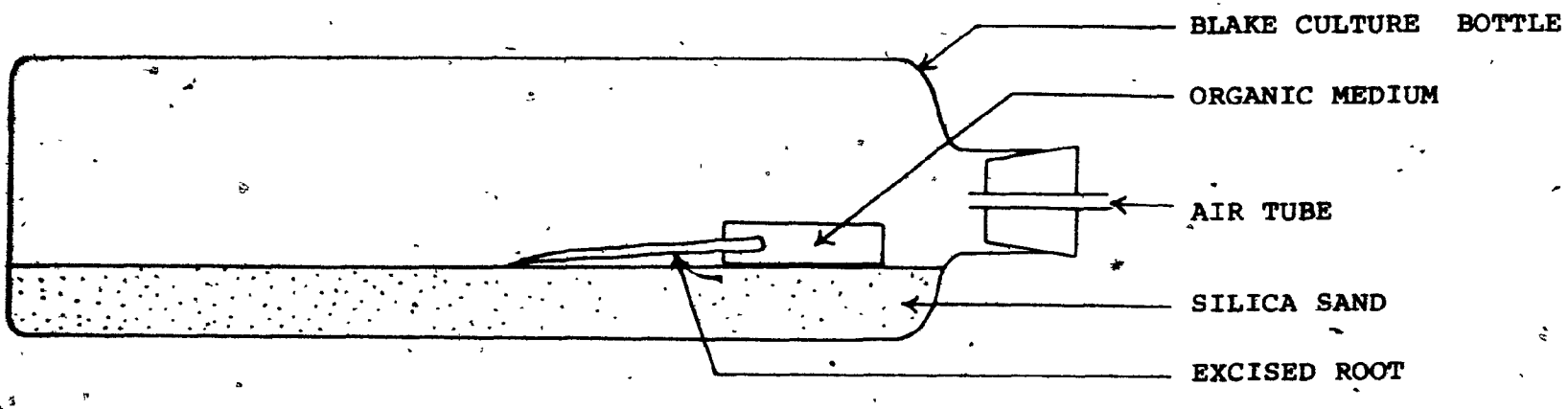


Figure 3. Diagram of culture bottle used in growth of excised bean roots.

placed on a segment of dry sterile filter paper to remove excess water. The inoculum disc was immediately transferred to a culture bottle and embedded in a narrow slit in the silica sand, previously made with a sterile needle.

Treatment three consisted of excised roots and an inoculation disc containing a drop of R. phaseoli. The bacterium was grown in a liquid medium on a laboratory shaker apparatus. The medium contained the following chemicals: mannitol, 10 g/l; yeast extract, 1.0 g/l; K_2HPO_4, 500 mg/l; $MgSO_4$, 200 mg/l; NaCl, 100 mg/l; $CaCl_2$, 200 mg/l; and distilled water, to 1,000 ml.

In treatment four, 1,000 Cephalobus sp., cultured on R. phaseoli (as described above for Chiloplacus sp.) were prepared for filter paper inoculation discs as in treatment two of this experiment.

Aseptic methods were used throughout the preparation of nematode and rhizobial inocula, root excisement, and final assembly of the different units.

All treatments, each replicated six times, were randomized and placed in a dark incubator at 24°C. The experiment was terminated after twenty-seven days. At this time the root systems were carefully removed from each Blake culture bottle and the number of nodules determined.

(ii) Results

The results of this experiment are shown below in Table 8.

Table 8. The effect of Cephalobus on the movement of R. phaseoli into the rhizosphere of excised bean roots

Treatment	Number of Nodules per Root Replicate 1	2	3	4	5	6	Total	Average
1. Uninoculated control	0	0	0	0	0	0	0	0^{b}[1]
2. Cephalobus sp. cultured on E. coli	0	0	0	0	0	0	0	0^{b}
3. R. phaseoli	0	0	5	14	4	0	23	3.8^{ab}
4. Cephalobus sp. cultured on R. phaseoli	41	3	4	18	2	6	78	12.3^{a}

[1]Any two means in a column with the same superscript are not significantly different (P 0.05).

Using the above technique of inoculation and excised root culture, it can be seen from the data in Table 8 that nodulation of excised roots by R. phaseoli alone was not prevented. Through self motility or passive flow in films of moisture, cells of R. phaseoli were able to move from the 10 mm inoculation discs, placed behind the excised root-containing vial of "organic" agar, to sites of nodulation along the root system. Effective restriction of this movement may not be possible in the required moist root environment.

It may be noted, however, that evidence for the transfer of rhizobial cells by saprozoic nematodes, into the rhizosphere of legumes, is clearly revealed by the data in Table 8. All replicates of treatment four, in which Rhizobium-fed Cephalobus sp. were added to excised bean roots, formed nodules. This

suggests that Cephalobus sp. migrated away from the inoculation disc toward the growing excised root system, and deposited viable cells of R. phaseoli at nodulation sites along the roots. Differences between the R. phaseoli treatment mean and the Cephalobus sp. plus R. phaseoli treatment mean were not significant (Appendix Table II).

4. Influence of Saprozoic Nematodes on Nodulation of Beans

In section one it was shown that saprozoic nematodes were capable of feeding on different species of Rhizobium in pure culture. In view of this, and since it is generally conceded that Rhizobium spp. (Alexander, 1961; p.331) and saprozoic nematodes (Daubert, 1959, cited by Katznelson, 1965; Henderson and Katznelson, 1961; Rouatt et al., 1963) are common inhabitants of legume rhizospheres, it may be assumed that, along with other bacteria, these saprozoic nematodes are feeding on the Rhizobium spp. From the reasoning of Yeates (1971), who commented that while Aphelenchus avenae is normally considered a mycophagous nematode, it could be termed plant parasitic in situations where mycorrhizae are attacked, it is possible to infer that saprozoic nematodes can debilitate legume plant growth through the reduction of Rhizobium population levels in soil. Therefore the relationship between saprozoic nematodes, such as Rhabditis sp., and Rhizobium sp. raises an important question: can Rhabditis sp., feeding on a population of R. phaseoli cells in the root zone of beans, alter significantly the rhizobial

population so that nodulation is affected? In order to answer this question, experiments were designed to determine what effect, if any, Rhabditis sp. had on the number of nodules formed on beans by R. phaseoli.

a. Experiment One

The objective of this experiment was to determine the effect of Rhabditis sp. on nodulation of beans grown in steamed soil in clay pots.

(i) Methods and Materials

A suspension of R. phaseoli cells was prepared by adding 20 ml of sterile distilled water to five-day old CRMA slants of R. phaseoli and shaking violently. A five ml suspension was used to inoculate the appropriate roots of the respective treatments described below.

The saprozoic nematode used in this experiment was an undetermined species of Rhabditis. Large numbers were prepared by culturing the nematodes on unknown bacteria on Corn Meal Agar. When sufficient numbers had developed, the nematodes were extracted through Baermann funnels, collected, and washed five times in sterile distilled water. Approximately 4,500 nematodes were pipetted in one ml amounts to each replicate of the appropriate treatment.

The cultivar of bean used was Slendergreen. Seeds were soaked in water, surface sterilized in 0.1% $HgCl_2$ for five minutes, and after rinsing in sterile water, plated on sterile

moist filter paper in Petri dishes. Only normal germinated seeds were selected for planting. One seedling was added to each of 36 ten cm clay pots of steamed sandy loam soil.

Steaming was carried out on two successive days, 30 minutes the first day and 90 minutes the second day. In order to remove potential toxic substances formed as a result of the steam treatment, the soil was leached with sterile tap water three times. The moist soil was left for 4 days on a greenhouse bench to dry.

The treatments were as follows:

1. Control
2. R. phaseoli
3. Rhabditis sp.
4. R. phaseoli and Rhabditis sp.

A depression was made in the soil of each pot and the inoculum added immediately prior to the seedling. The soil was replaced over the seedlings and tamped down firmly. Each pot was placed on a sterile saucer and randomized on a greenhouse bench. Watering was done with sterile tap water. The plants were illuminated by sunlight supplemented with 15,000 lx of artificial light for a daylength of 16 hours. The experiment was conducted for 11 weeks, at which time the plants were harvested and the number of nodules determined.

(ii) Results

The results of this experiment are summarized in Table 9.

Although nodules formed on uninoculated control plants, and two replicates of the R. phaseoli treatment did not grow, the data in Table 9 suggest that Rhabditis sp. may reduce the population of R. phaseoli. The number of nodules in plants treated with R. phaseoli and Rhabditis sp. were 32% fewer than in those treated with R. phaseoli alone. This data is somewhat confusing because of the greater (28%) number of nodules formed in the Rhabditis sp. treatment compared to the uninoculated control. In this latter case, the nematodes were probably acting to disseminate the rapidly dividing rhizobia, hence a greater average number of nodules. Because of the nodulation of uninoculated control roots and Rhabditis sp. treated roots, the data were not analyzed statistically.

The appearance of nodules in the uninoculated control treatment was unexpected. Tests were conducted to determine whether viable cells of R. phaseoli were present in the atmosphere and in soil after steam treatments of the soil. The results were negative in all tests.

Bean plant growth was normal, with the production of dark green foliage and bean pods at the termination of the experiment. Plant height was variable, but there was little difference between treatments regarding dry weight.

The number of nematodes was determined when the experiment was completed. In both treatments where Rhabditis sp. was added, there was a slight increase in the number of nematodes between inoculation and harvest. The numbers were not, however, much greater than the initial inoculum, nor were they

different between treatments. It appears, therefore, that in the Rhabditis sp. treatment, bacteria introduced by the nematodes multiplied and served as a source of food for the nematodes.

Table 9. Effect of Rhabditis sp. on nodulation of Slendergreen beans by R. phaseoli

	Treatment			
Replicate	Control	R. phaseoli	Rhabditis sp.	R. phaseoli & Rhabditis sp.
1	142[a]	66	231	43
2	48	162	18	34
3	102	224	55	91
4	69	-[b]	87	59
5	115	149	29	79
6	27	131	95	197
7	86	172	245	-[b]
8	78	-[b]	112	113
9	97	120	107	169
Total	764	1024	979	805
Average	84.7	146.3	108.7	100.6

[a]Figures represent total number of nodules per plant.

[b]Plants failed to grow.

b. Experiment Two

Due to the formation of nodules in the uninoculated control treatment in the above experiment, it was decided to eliminate soil and exposed clay pots by using perlite

and enclosable glass vessels. The objective of this experiment, therefore, was to determine the effect of a large population of Rhabditis sp. on the nodulation of beans grown in nutrient soaked perlite in special 500 ml Erlenmeyer flasks.

(i) Methods and Materials

The apparatus used to grow the bean plants was a rubber stopper enclosed 500 ml wide-mouth Erlenmeyer form flask. Each of twenty-four flasks was filled with dry perlite and fitted with a Number 10 rubber stopper containing an air tube (outside diameter 6 mm, length 175 mm), two inoculation tubes (diameter 6 mm, length 75 mm) and a planting tube (diameter 20 mm, length 75 to 80 mm). The entire length of the air tube was packed with non-absorbent cotton. The completed units were autoclaved for 15 minutes at 101.3 kPa. Using aseptic technique, 200 ml of a sterile nutrient solution (Piper, 1941; Mulder, 1948; and Hewitt, 1952) suitable for the growth of legumes, were added to each flask. This modified nutrient solution contained the following chemicals: K_2HPO_4, 200 mg/l; KH_2PO_4, 200 mg/l; $Na_2HPO_4 \cdot 2H_2O$, 50 mg/l; ferric citrate, 25 mg/l; $MgSO_4$, 250 mg/l; $CaSO_4$, 250 mg/l; $MnSO_4$, 1.0 mg/l; $ZnSO_4$, 0.25 mg/l; $CuSO_4$, 0.25 mg/l; H_3BO_3, 0.25 mg/l; Na_2MoO_4, 0.25 mg/l and distilled water to 1,000 ml. Nitrogen was omitted from the medium. In order to avoid precipitation of salts, different components of the medium were autoclaved separately. The phosphates (combined) and iron were dissolved separately in 100 ml of distilled water

and autoclaved for 15 minutes at 101.3 kPa. The rest of the chemicals (mainly sulphates) were dissolved in 800 ml of distilled water and autoclaved. All three groups were combined, using aseptic transfer technique, when the solutions had cooled to room temperature. Little precipitation occurred using this method. The final pH value of the nutrient solution was 7.5.

Slendergreen bean seeds were water soaked for 5 hours, surface sterilized in a solution of 0.1% $HgCl_2$ for 5 minutes, rinsed in sterile water and plated on CRMA. Germinated seedlings were selected and transferred, one per flask, to the planting tubes. This was done by detaching the planting tube and inserting the seedling until the radicle contacted the moist perlite. The planting tube was then replaced and the seedling covered with sterile moist perlite. Each flask was wrapped with aluminum foil and placed in a growth chamber at a constant temperature of 26°C.

At four to six days the bean cotyledons had emerged and the planting tubes were filling with the elongating growth of the plants. At this time the units were returned to the laboratory for inoculum treatments.

The treatments, each replicated six times, were as follows:

1. Control
2. R. phaseoli
3. Rhabditis sp.
4. R. phaseoli and Rhabditis sp.

In treatment one, the planting tubes were removed and the exposed area between the hypocotyl and edge of the rubber

stopper filled with petroleum jelly. This served to prevent not only microbial contamination of the root system and water loss, but also allowed for expansion of the growing stem. In all other treatments, petroleum jelly was applied immediately after inoculation.

In treatment two a 10 ml suspension of R. phaseoli, prepared as in experiment one, was added around the base of the hypocotyl region.

In treatment three, a five ml suspension of Rhabditis sp. (containing approximately 3,700 nematodes), prepared as in experiment one, was added in the same way as was the R. phaseoli.

Treatment four consisted of both R. phaseoli and Rhabditis sp. inoculations.

When the treatments had been applied the flasks were returned to the growth chamber and adjustments were made for daylength and temperature (described below). Due to the extreme moistness of the rooting medium and slight aeration provided by the inoculum and air tubes, the plants were given 30 minutes of forced aeration everyday. This was accomplished by fitting rubber tubing to all flasks and pumping air slowly through the cotton-packed air tube. An automatic electric time switch was used to regulate the desired length of the aeration.

The plants were given 20,000 lx of artificial illumination for a period of 16 hours per day. This was followed by an 8 hour dark period.

At five-day intervals freshly prepared sterile medium was added to each unit to replace moisture lost from the rooting medium due to vigorous plant growth. The medium was dispensed to each unit through the inoculum tubes using a sterilized, automatically refilling, syringe pipettor.

The experiment continued for 23 days. On the twenty-third day, the roots were harvested, carefully washed, and the number of nodules counted.

(ii) Results

The results of this experiment, in which the effect of Rhabditis sp. on the nodulation of beans was determined, are summarized in Table 10.

It is immediately evident that successful control was obtained over contamination of the uninoculated control treatment by R. phaseoli. Because nodule formation did not occur, and nitrogen was omitted from the nutrient solution, plant growth in this treatment exhibited typical nitrogen deficiency symptoms. Terminal leaflets were light green in colour whereas the lower leaves ranged from a lightgreen to a mottled dark green with yellow-orange borders and were easily dislodged from the plant.

The data in this experiment were not analyzed statistically due to the appearance of nodules in the Rhabditis sp. treatment. Nodulation of roots treated with Rhabditis sp. indicates that the species composition of the unknown bacteria used to culture Rhabditis sp. included R. phaseoli. It should be pointed out

that it has been claimed no Rhizobium spp. is capable of nodulating members of the bean group, Phaseolus vulgaris, other than R. phaseoli (Alexander, 1961). Compared with the number of nodules in the R. phaseoli treatment, or the R. phaseoli plus Rhabditis sp. treatment, there were appreciably fewer nodules formed on the plants that were presumed to have been treated by Rhabditis sp. alone. This was most likely due to the initially low number of viable R. phaseoli cells in the population of bacterial cells introduced into the root zone by Rhabditis sp. Despite nodule formation, deficiency symptoms similar to those in the uninoculated control treatment were apparent. Average dry weight measurements of plant growth in the Rhabditis sp. treatment (foliage, 0.33 g; roots, 0.18 g) were nearly indentical to those in the uninoculated control (foliage, 0.30 g; roots, 0.18 g).

The figures for nodulation in treatments two and four are approximately similar, although slightly more (359.6) nodules were formed in the R. phaseoli and Rhabditis sp. treatment than in R. phaseoli alone (341.5). This slight difference in nodulation between treatments may have been due to the R. phaseoli cells introduced with the nematode inoculum. Dry weight measurement of plant growth in the R. phaseoli treatment (foliage, 0.39 g; roots, 0.25 g) compared with the R. phaseoli and Rhabditis sp. treatment (foliage, 0.50 g; roots 0.23g)

Table 10. Effect of Rhabditis sp. on nodulation of Slendergreen beans by R. phaseoli

Replicate	Treatment			
	Control	R. phaseoli	Rhabditis sp.	R. phaseoli & Rhabditis sp.
1	0[a]	332[a]	60[a]	423[a]
2	0	295	116	327
3	0	438	84	445
4	0	-[b]	55	217
5	0	301	28	-[b]
6	0	-[b]	-[b]	386
Total	0	1,366	343	1,798
Average	0	341.5	68.6	359.6

[a]Number of nodules per plant.

[b]Plants failed to grow.

revealed a 28.2% greater foliage growth in the combined inoculum treatments than in R. phaseoli alone. In both treatments the terminal leaves were dark green while the lower, older leaves were also dark green with narrow, chlorotic borders.

Plant growth in R. phaseoli or combined R. phaseoli plus Rhabditis sp. treatments was greater than in either the control or Rhabditis sp. treatment. R. phaseoli treated plants showed 38.8% greater root growth than the control or Rhabditis sp. treatments, whereas in the combined inoculum treatment, root growth was 27.7% greater than control or Rhabditis sp. alone.

Foliage dry weight in R. phaseoli treated plants was 30% and 18.2% greater than control or Rhabditis sp. treatments, respectively. However, in comparison with the R. phaseoli treatment alone, foliage dry weights for the combined inoculum treatment were even greater with 66.6% and 51.5% more growth being produced than control or Rhabditis sp. respectively.

5. Effect of Temperature on the Hatching of Saprozoic Nematode Eggs

Temperature effects on certain biological aspects of saprozoic (free-living) nematodes have been well documented. Nielsen (1949b) found that apart from the age of the nematode, temperature was the factor which caused the most profound alterations in the respiratory rate of saprozoic nematodes. He found that as the temperature increased from 13°C to 28°C, the oxygen consumption of Mononchus papillatus and Plectus cirratus doubled. Pillai and Taylor (1968) noted that temperature influenced the time required for egg hatch of Paroigolaimella berensis and Fictor anchicoprophaga. The effect of temperature on the length, body proportions and rate of reproduction of some rhabditid nematodes was studied by Sohlenius (1968). However, the effect of temperature on the hatching of eggs of rhabditid nematodes has not been determined. Accordingly, the experimental work described below reports on the effects of temperature on egg hatch of a Rhabditis sp. (spec. indet.).

a. Methods and Materials

Eggs were obtained from the R1 isolate of Rhabditis sp. reared on a mixture of bacteria on corn meal agar. The eggs were surface sterilized in 0.05% NaOCl and transferred to sterile disposable Petri dishes (60 mm diameter, 15 mm height) in five ml of sterile distilled water. Fifteen eggs were transferred to each plate.

The plates were placed in four temperature controlled incubators located in a 4°C cold room. Temperatures used for this study were 15, 20, 25 and 30°C. Each treatment was replicated four times. To determine whether nutrients in solution, compared with distilled water, would promote egg hatch, one plate containing 5 ml of a simple nutrient solution was included at each temperature. The composition of the medium was as follows: mannitol, 10 g/l; K_2HPO_4, 500 mg/l; $MgSO_4 \cdot 7H_2O$, 200 mg/l; NaCl, 100 mg/l; $CaSO_4$, 200 mg/l; yeast extract, 1.0 g/l and distilled water to 1,000 ml (Harrigan and McCance, 1966).

The number of hatched juveniles were counted at 12, 36 60 and 96 hours using a Wild M-5 stereomicroscope. The experiment was terminated after 96 hours.

b. Results

The results of this experiment are summarized in Table 11 and Figures 4 and 5.

The effect of temperature on egg hatch is clearly revealed in Table 11 and Figure 4. The number of hatched juveniles

increased with temperature, the best temperature of this test series being 25 to 30°C. Differences in egg hatch were not statistically significant (Appendix Table III). The highest and lowest temperature at which egg hatch would occur was not determined. Fifteen degrees centigrade strongly reduced egg hatch with only three eggs out of sixty producing juveniles (Table 11).

Table 11. Effect of temperature on egg hatch of Rhabditis sp.

Temperature	Medium	Number of Eggs Hatched Time in Hours 12	36	60	96
15°C	Dist. water	0.25[2]	0.50	0.75	0.75[a][1]
	Nutr. solution	0	0	1	5
20°C	Dist. water	0.75	0.75	1.25	2.25[a]
	Nutr. solution	0	5	5	7
25°C	Dist. water	1.00	2.00	3.50	4.25[a]
	Nutr. solution	1	9	12	12
30°C	Dist. water	1.50	4.50	4.50	4.50[a]
	Nutr. solution	6	11	13	13

[1]Any two means in a column with the same superscript are not significantly different (P≤0.05).

[2]Average of four replicates.

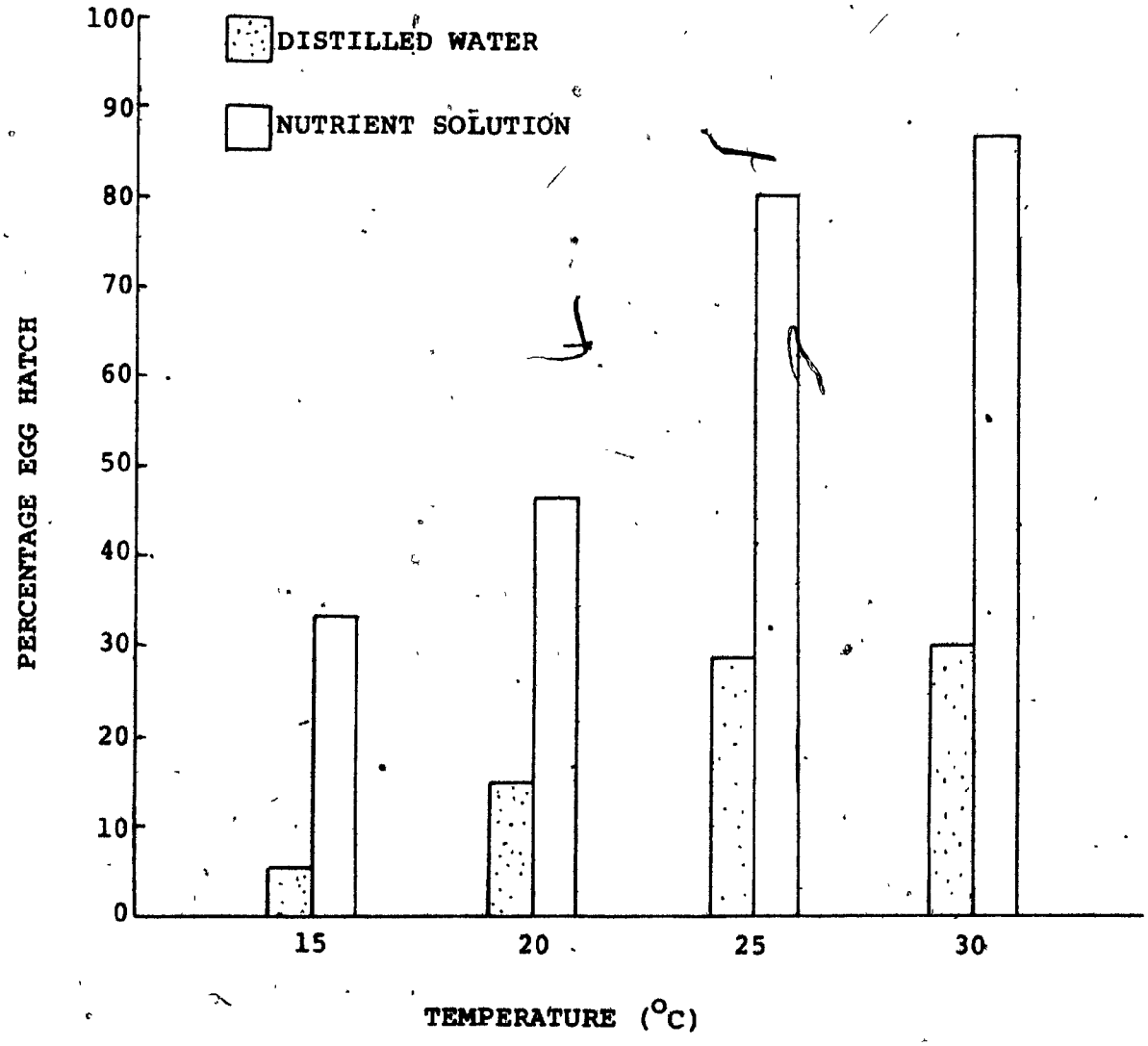


Figure 4. Effect of temperature on egg hatch of isolate R_1 of Rhabditis sp. in distilled water and in a nutrient solution, at 96 hours.

Stimulation of egg hatch by nutrients in the medium is clearly seen in Figure 4. At 15, 20, 25 and 30°C, egg hatch was increased 28, 32, 52 and 57% in nutrient solutions compared with distilled water. It must be kept in mind, however, that the percentage values obtained for egg hatch in distilled water are derived from averages of four replicates whereas only one replicate represents the values for hatched eggs in nutrient solution. The effect of nutrients in the medium appeared to overcome the suppressive effects of the 15°C temperature with egg hatch reaching 33% in the nutrient solution compared to 5% in distilled water. This effect was so pronounced that there was a greater egg hatch at 15°C in nutrient solution (33.3%) than at 30°C in distilled water (30%). In this series of tests the optimal temperature for egg hatch in nutrient solution was 25 to 30°C, the same value as in distilled water.

The hatching response of isolate R1 of Rhabditis sp. at different times is shown in Figure 5. In distilled water, optimal egg hatch at 30°C was reached after 36 hours of incubation whereas 60 to 96 hours were required for a similar response at 25°C. The curves plotted for egg hatch at 15 and 20°C were much less steep than those for 25 and 30°C, indicating a considerably slower response at these temperatures.

Figure 5B depicts the results of egg hatching in a simple nutrient solution. The hatching response at 30°C was rapid with 40% of the eggs producing motile juveniles within 12 hours.

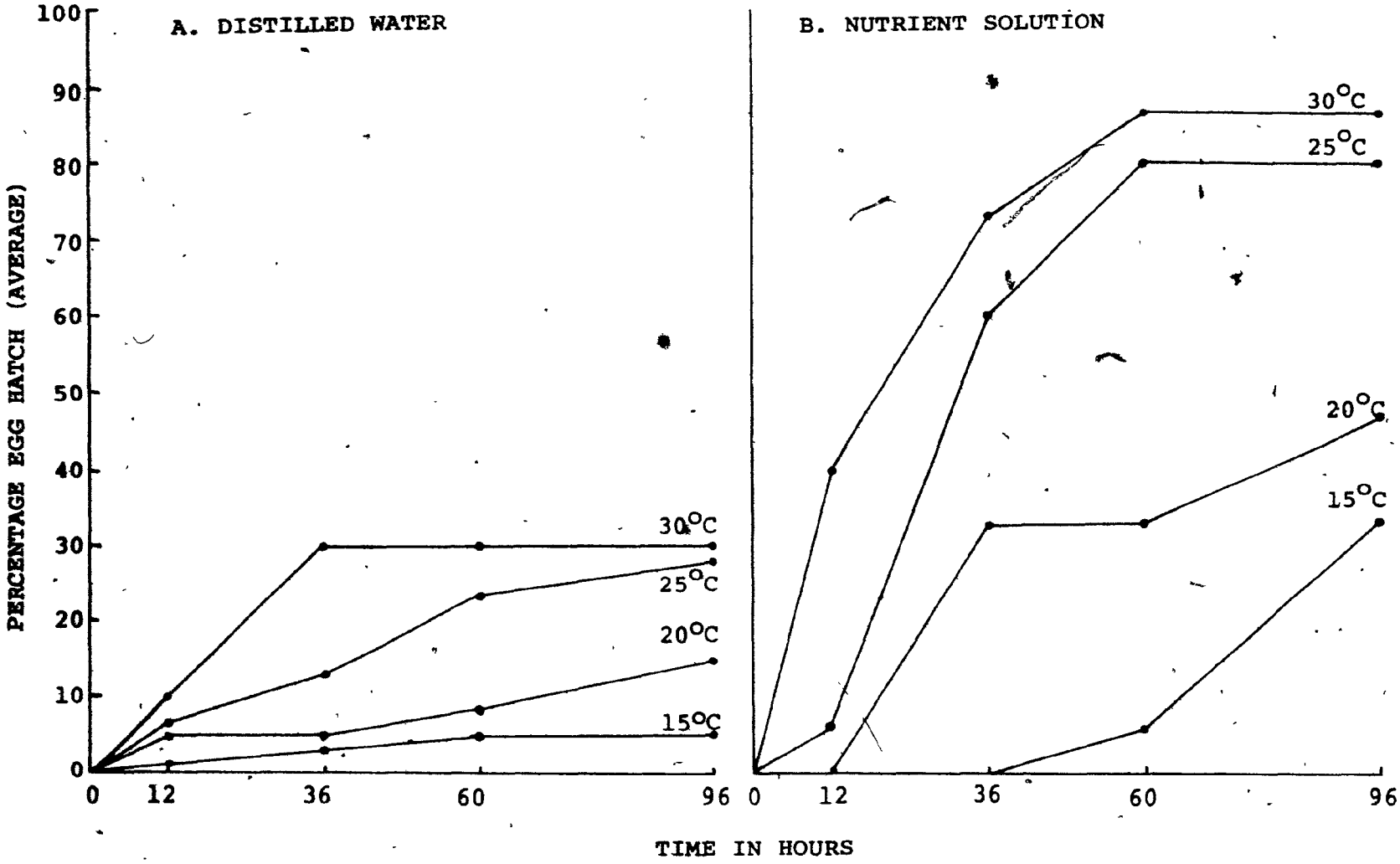


Figure 5. Effect of temperature on egg hatch of Rhabditis sp. (isolate R_1) at 12, 36, 60 and 96 hours.

Hatching continued to occur rapidly until 60 hours after which the maximum number of eggs had hatched. Except for an initially slow response, the curve for egg hatch at 25°C is identical to that of 30°C. At 20°C no eggs had hatched in the first 12 hours of incubation compared with 5% egg hatch in distilled water by this time. However, hatching occurred rapidly after 12 hours and continued until 36 hours after which the curve levelled off. Egg hatch was strongly inhibited at 15°C until the thirty-sixth hour. Afterwards, the appearance of hatched juveniles took place slowly until 60 hours, and rapidly thereafter.

As a result of the necessity to remove the lids of the Petri dishes for accurate counting of hatched juveniles, the nutrient media became contaminated. After 24 hours the 25 and 20°C treatments were turbid. The other temperature treatments remained visually uncontaminated but probably contained microbial cells. No attempt was made to verify this using bacteriological testing. Hatched juveniles in dishes of nutrient solution remained active throughout the experiment whereas those in distilled water were quiescent and appeared dead. Microbial exudates, in the nutrient solution, may have stimulated the eggs to hatch. The nutrient solution, therefore, may have been only indirectly stimulatory.

DISCUSSION AND CONCLUSIONS FOR PART I

The capability of different saprozoic nematodes to ingest, grow, and develop to maturity on different species of Rhizobium, has been shown to occur using monoxenic cultural conditions. These findings represent the first recorded observations concerning growth of freeliving nematodes on Rhizobium spp. Nielsen (1949b) reported the growth and development of several saprophagous nematodes, under synxenic conditions, on a mixed bacterial culture derived from root nodules of Trifolium. Although the presence of Rhizobium trifolii would be suspected in the mixture, it is not possible to ascertain with certainty which bacterium the nematodes were consuming and utilizing for nutrients. The only parallel study in the literature is that of Singh (1942) who observed that of 16 strains of Rhizobium added to cultures of protozoa, none were consumed by either a soil flagellate or two amoebae.

The growth of freeliving nematodes on Rhizobium, a gram negative rod-shaped bacterium, is in agreement with the generalized conclusion, presented by Dougherty and Calhoun (1948) and supported by Sohlenius (1968) and Tietjen et al., (1970), that gram negative rod-shaped bacteria are more suitable than gram positive bacteria as food for rhabditid forms. Contrasting evidence, however, has been recorded by Nielsen (1949b) and Yeates (1970) who found that several gram positive rods and cocci supported ample growth of freeliving nematodes.

The length of time required to develop from egg to egg was approximately 12 to 15 days, which agrees with the results

of Nielsen (1949b). There appeared to be little difference in growth rate between nematode species on the different Rhizobium isolates. Although only one type of medium (CRMA) was used to follow freeliving nematode development on Rhizobia from egg to egg, Sohlenius (1968) noted that the type of medium used in saprozoic nematode culture affects their growth, development and reproduction. Yeates (1970) suggested that differences in nematode reproduction on different media reflected quantitative changes in the bacterial flora, which presumably was due to variation in nutrient availability of the different media.

In the first experiment, in which a diluted Nutrient Agar medium was used, the morphology of the Rhizobium spp. was observed periodically. Examination revealed the presence of normal rod-shaped Rhizobium cells and much larger, more predominant Y, L, star, oval and club shaped bacteroid forms. Initially, these unusual forms were considered as contaminants. Bacteroid forms of Rhizobium were found by Jordan and Coulter (1965) to be caused by the amount of yeast extract in the medium. In this experiment, therefore, two conclusions are apparent: (1) that standard Nutrient Agar, which does not contain yeast extract, must contain other nutrients (probably proteins) which promote bacteroid formation and, (2) since there were very few normal rod-shaped rhizobial cells present, saprozoic nematodes can utilize the bacteroid forms of Rhizobium for their nutritional requirements.

Hatched juveniles on control plates survived the entire incubation periods for both experiments, namely 14 and 21 days. These results are not in accord with Nielsen (1949b), who used different species of nematodes. He found that newly hatched juveniles died after 7 to 10 days in sterile water or sterile soil suspensions. Differences between the above results and those of Nielsen (1949b), may also reflect the use of different experimental techniques. Nielsen used petroleum jelly-sealed hanging drop moist chambers on slides, which probably retarded the diffusion of oxygen and led to a relatively early death of the nematodes. The Petri dishes used in the experiment above, allowed adequate amounts of oxygen, necessary for these highly aerobic organisms (Nicholas and Jantunen, 1966). That juveniles survived for such considerable periods of time may be due to various factors, including inner food reserves, low food requirements, and moisture supplied by the medium.

Normal growth and development of a Rhabditis sp. (isolate R_2) took place in monoxenic culture on five different yeasts. These results are in agreement with Hopper and Meyers (1966,a,b) who reported feeding and reproduction of two freeliving marine nematodes on marine yeasts, and Yuen (1968), who cultured Panagrellus silusiae on agar-grown yeasts. It should be noted, however, that Hopper and Meyers (1966a,b) used as inoculum nematodes isolated from a fungus-infested cellulose matrix. No attempt was made to free these nematodes of external or internal contaminating bacteria. Also Yuen (1968) gave no details

regarding the culture of Panagrellus silusiae on agar-grown yeast. Therefore, in the latter two studies, it is highly probable that freeliving nematode growth was primarily due, not to the yeast cells, but to smaller, more easily ingested bacterial cells. McCoy (1929) and Nielsen (1949b) were unsuccessful in attempts to culture juvenile saprophagous nematodes on yeasts.

Growth and reproduction of freeliving nematodes on yeasts in culture may reflect important interactions between these two groups in soil and in the rhizosphere. Some yeasts, such as Rhodotorula spp. and Torulopsis spp. are found in large numbers only in the rhizosphere of certain plants (Babeva and Belyanin, 1966). Since rhizosphere yeasts have been found to liberate appreciable amounts of amino acids (Babeva and Saveleva, 1963), it is conceivable that these compounds might not only play a role in supplying plants with biologically active compounds, as suggested by Babeva and Saveleva (1966), but also the rhizosphere microflora, including saprozoic nematodes and bacteria. Metcalfe and Chayen (1954) discovered two isolates of soil yeasts, one a Rhodotorula sp. and the other a Saccharomyces sp., capable of fixing atmospheric nitrogen. The Rhodotorula sp., which was found to have nitrogen fixing capacities greater than the Saccharomyces sp., was shown by Roberts and Wilson (1954) to be capable of fixing molecular nitrogen at one-tenth capacity of Azotobacter, a well-known nitrogen fixing aerobic

bacterium. Ingestion by nematodes therefore, of soil yeasts capable of assimilating molecular nitrogen, and rhizosphere yeasts, which liberate amino acids, may be of significant nutritional importance.

The rate of reproduction as measured by the time required to develop from egg to egg was longer for yeast-cultured Rhabditis sp. (isolate R_2) than for the same nematode on Rhizobium spp. Vigorous egg production occurred after 18 days on most yeasts whereas Rhizobium spp. supported rapid production after only 15 days. Of the five yeasts tested Rhodotorula pallida supported the most rapid growth and development of the R_2 isolate of Rhabditis sp., which reached the adult stage and started producing eggs after only 12 days. On Saccharomyces cerevisiae however, adulthood was not reached until the twenty-first day, at which time egg deposition began.

Migration downward through surface inoculated sterile 50 mm soil columns, of a Rhabditis sp. (isolate R_2), was shown by recovery of the nematodes at various depths in the column after incubation periods of 12, 24, 36, 48 and 60 hours. Capability of moving several centimetres in a short time period was indicated by retrieval of 46.1, 22.4, 18.4 and 13.1 percent in the first, second, third and fourth portions, respectively, of the soil column after 12 hours. During this early incubation period, only 46 percent of the nematodes remained near the top of the column, whereas 54 percent migrated into the lower zones. As

the incubation time increased, however, the greatest numbers of nematodes were extracted from the uppermost portions of the soil column, indicating that, after the initial downward migration, there was a reversal of nematode movement. It would appear that following inoculation of the soil surface, the nematodes, which previously had been cultured on a mixture of bacteria and given several rinses in sterile water, migrated downward through the soil column in search of food, or they may have done so in response to gravity. In the course of movement among the moist soil particles, one may assume that adhering bacteria were removed and internal viable cells were excreted. The dislodged or defecated bacteria would multiply on the fresh, uncolonized substrates in the sterile soil. These bacteria, being aerobic, could be expected to grow more rapidly in the uppermost portion of the tubed soil and to serve as food sources for the retreating nematodes. It is reasonable to assume, therefore, that in seeking a highly oxygenated environment (Nicholas and Jantunen, 1966), the nematodes were also attracted to the rapidly dividing bacteria.

Transport of Rhizobium phaseoli cells into root zones of Phaseolus vulgaris by different freeliving nematodes, was consistently demonstrated by the appearance of nodules several centimetres distant from the point of inoculation. Nodules also formed in some roots of the R. phaseoli treatments in the absence of nematodes. This indicates either: (1) movement of

the motile bacterium along moisture films to the roots, (2) growth of the roots into areas occupied by the bacterium, or (3) both of the above. Inasmuch as the average number of nodules counted in nematode plus R. phaseoli treatments was always greater than R. phaseoli treatments alone, as indicated by increases of 84%, 9% and 224% in the three experiments performed, it is clear that the nematodes were responsible for most of the rhizobial dissemination. Nematode transport of rhizobia in soil, therefore, may contribute in a significant, and perhaps immeasurable way, to the nitrogen economy of the soil.

These results are in agreement with the concepts of Jensen (1967) who theorized, on the basis of laboratory evidence, that freeliving nematodes could convey plant pathogenic microorganisms within plant rhizospheres and from one rhizosphere to another. Soloveva (1965) supported Jensen's view by showing that spores of Plasmodiophora brassicae were carried by Panagrolaimus rigidus from sites of inoculation into vulnerable areas along cabbage roots. Soloveva noted that in the P. brassicae treatment alone, only plants close to the site of inoculation were infected, whereas in the combination with fungus and nematodes, all plants showed clubroot disease symptoms.

An important point of consideration is that not only do freeliving nematodes transport Rhizobium spp. to legume rhizosphere and rhizoplane areas directly, but because of random dissemination of the rhizobial inoculum during nematode movement in the soil and widespread growth of the plant root system, contact between both symbiotic associates is indirectly

facilitated. Also, it should be remembered that since free-living nematodes can utilize different Rhizobium spp. as sources of food for growth, many of the transported cells would be digested and destroyed (Nielsen, 1949b; Alexander, 1971) or chemically damaged by the digestive processes of the nematodes (Jensen and Stevens, 1969).

On the basis of his extensive soil studies, Nielsen (1949b) concluded that the ecological significance of bacteriophagous nematodes in soil is twofold: (1) bacterial numbers and total bacterial activity are reduced due to the food requirements of the nematodes, and (2) multiplication of the nearby non-ingested bacterial population occurs due to the availability of mobilized nutrients contained in the dead bacterial protoplasm, and space occupied by the previous bacteria. Nielsen, however, did not determine bacterial population decreases or increases, but merely generalized on the basis of other studies dealing with bacteria and protozoa. In addition to Nielsen's conclusions regarding the importance of bacterial feeding nematodes, a third may now be added based on the studies above concerning conveyance of cells of R. phaseoli by freeliving nematodes to nodulation sites along roots of beans: (3) bacteria (or any ingested or surface adhering microorganisms) are actively distributed throughout soil due to random or directional movements of the nematodes brought about by attraction-repulsion mechanisms of plant roots or members of the microflora.

Although Nielsen (1949b) and other workers (Dougherty and Calhoun, 1948; Sohlenius, 1968; Alexander, 1971) have suggested that total numbers and activity of soil bacteria decline in the presence of bacteriophagous nematodes, a slight increase in the number of R. phaseoli cells (as indicated by nodule number) occurred in Rhabditis sp. and R. phaseoli interactions (Table 10), compared with the R. phaseoli treatment alone. One of several tenable explanations of the above is that in addition to the R. phaseoli inoculum deliberately added, the Rhabditis sp., which was cultured on a mixture of unknown soil bacteria, carried a strain of R. phaseoli capable of infecting and nodulating the roots of beans. This strain of R. phaseoli, which nodulated bean roots treated with Rhabditis sp., may be categorized as in ineffective root nodule bacterium (Alexander, 1961), since not only were the nodules small and widely distributed over the root system, but the plants were chlorotic indicating inability to assimilate molecular nitrogen. Therefore, despite the feeding activities of the Rhabditis sp., and the subsequent depressing effects this would have on the population of both groups of R. phaseoli cells, the number of nodules in both treatments were approximately the same because of the additional R. phaseoli inoculum carried by the nematodes.

It is also possible that, due to the vast number of cells introduced in the 10 ml R. phaseoli inoculum, and in spite of the amount consumed by the Rhabditis sp., no significant

depressing effect occurred in the Rhizobium sp. population. Hence, the number of nodules remained approximately the same in both treatments. Furthermore, since it has been demonstrated that freeliving nematodes defecate viable bacteria when they consume bacteria in excess of their digestive capacity (Chantanao and Jensen, 1969b), and because the present study has shown that freeliving nematodes can transport R. phaseoli cells into root zones of beans, it is highly probable that Rhabditis nematodes ingested R. phaseoli cells and during migratory movements, transported the bacteria to new nodulating sites along the roots. As a result of this a slightly greater number of nodules formed in the combined inoculum treatment than in the Rhizobium treatment alone.

Temperature, and the nature of the suspending medium, strongly affected the hatching response of eggs from a Rhabditis sp. (isolate R_1). The number of newly emerged juveniles increased as the temperature increased from 15 to 30°C (Figure 4). This is comparable to the results of Pillai and Taylor (1968) who found that the time required for the hatching of eggs of two freeliving nematodes decreased as the temperature increased, and also supports the conclusion of Nielsen (1949b) that temperature affects growth and reproduction of freeliving nematodes significantly.

Egg hatchment not only responded to temperature changes but was affected by the chemical composition of the medium.

Compared with distilled water, in which hatching was inhibited, the presence of mannitol, K_2HPO_4, $MgSO_4$, NaCl, $CaSO_4$ and yeast extract in the suspending fluid appeared to indirectly stimulate egg hatch (Figure 4). Juvenile emergence from eggs in the contaminated nutrient solution was 33, 47, 80 and 87% at temperatures of 15, 20, 25 and 30°C, respectively. Egg hatch, calculated from the data of Yeates (1970), who worked with six different dune sand nematodes, ranged from 54% to 85% at 15°C and 47% to 81% at 20°C. The higher values of Yeates probably reflect the use of a solid agar medium allowing greater oxygen availability compared with the liquid medium above.

PART II

STUDIES OF APHELENCHUS AVENAE

1. Selective Preference of A. avenae for Different Host Fungi

A nematode frequently isolated from the rhizospheres of plants is Aphelenchus avenae Bastian, (Thorne, 1961; Jenkins and Taylor, 1967). Although evidence exists which suggests that, under certain conditions, A. avenae is a parasite of higher plants, it is commonly accepted that A. avenae obtains nutrients necessary for completion of its life cycle from the fungi (Goodey, 1965; Jenkins and Taylor, 1967). Published results of research indicate that this mycophagous nematode is not only able to reproduce on fungi, but, under certain conditions, can preferentially select its fungal host.

In order to determine whether A. avenae would preferentially select specific fungi in both agar and soil conditions, a number of experiments were designed. These are described on the following pages.

a. Experiment One

The objective of this experiment was to determine whether A. avenae would be attracted to colonies of different fungi using an agar medium.

(i) Methods and Materials

Twelve sterile Petri dishes (diameter 100 mm, height 15 mm) were poured with Difco PDA. When the medium solidified, three 7 mm discs cut with a sterile cork borer, were removed from each plate at approximately equal distances from the center. Each disc removed was equidistant from each other. Into each vacant space was placed a 7 mm disc of PDA on which one of the following fungi was growing: Trichoderma viride, Pers. ex Fr.; Sclerotinia sclerotiorum, (Lib.) de Bary; Verticillium albo-atrum, Reinke and Berth.; Verticillium dahliae, Kleb.; an unidentified species of Fusarium, (Link ex. Fr.) and Fusarium culmorum, (W. G. Sm.) Sacc. The fungi were arbitrarily placed in groups of three in the following way; Group A: T. viride, S. sclerotiorum and V. albo-atrum,; Group B: V. dahliae, F. culmorum and Fusarium sp. and Group C: control. There were four replicates per treatment. The inoculated plates were incubated at room temperature for 24 hours to allow for initial growth of each fungus into the surrounding PDA.

A. avenae was cultured on Sclerotium bataticola Taub. which was grown on PDA. The nematodes were extracted by means of Baermann funnels, washed for 5 min in 0.01% $HgCl_2$ and rinsed twice in sterile distilled water using a small laboratory centrifuge. The final volume of the A. avenae suspension was adjusted to 5 ml, and 0.1 ml amounts were pipetted onto the center of each plate of fungi. The number of nematodes in each

0.01 ml aliquot was not immediately determined, but after the plates had incubated at 24°C for 19 hours, the A. avenae within each fungal colony were counted with the aid of a wild M-5 stereomicroscope.

(ii) Results

From the results summarized in Table 12, it is clear that, of the six fungi tested, only T. viride, with an average of 13.5 nematodes per colony, was strongly and significantly preferred (Appendix Table IV) by A. avenae. By comparison, S. sclerotiorum was only slightly preferred by this nematode as just 4.5 nematodes (average) were found in each colony of this fungus. Very few A. avenae aggregated in colonies of F. culmorum and the unidentified Fusarium sp. indicating that these fungi were not found attractive by this nematode. Both colonies of the Verticillium species tested did not contain any nematodes. It must be pointed out, however, that in all four replicates of T. viride and in replicates two and four of S. sclerotiorum, fungal mycelial growth extended, at the time the experiment was terminated (43 hours), either immediately adjacent to the periphery of the inoculation zone or into this zone. Therefore, interpretation of the data is difficult as the nematode accumulation may have been due to a combination of attracting substance(s) and the rapid growth rate of the fungus. Another important factor to take into consideration

Table 12. Preferential selection of different soil fungi by A. avenae in a PDA medium

Fungus Grouping	No. of A. avenae Per Replicate in Fungus or Control Zone 1	2	3	4	Total	Average
Group A						
T. viride	31	5	4	14	54	13.5[a1]
S. sclerotiorum	3	2	4	9	18	4.5[ab]
V. albo-atrum	0	0	0	0	0	0[b]
Group B						
Fusarium sp.	2	0	5	1	8	2.0[ab]
F. culmorum	3	1	0	1	5	1.2[ab]
V. dahliae	0	0	0	0	0	0[b]
Group C						
Control	0	0	0	0	0	0[b]

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

is that despite efforts to surface sterilize A. avenae, a small number of contaminating bacteria and fungi (mainly S. bataticola) were present in the nematode inoculum. Consequently, the contaminants obtained nutrients from the PDA and multiplied. In several instances A. avenae was observed feeding on small mycelial strands in the nematode inoculation zone.

In the control plates, all nematodes added were located in the inoculation zone. Many were feeding on growing mycelial strands of S. bataticola which had survived the nematode surface sterilization treatment.

b. Experiment Two

In experiment one it was shown that A. avenae was attracted in largest numbers to only two of six fungi. In this experiment the number of fungi tested was increased and slight, but significant, changes were made in the agar medium.

(i) Methods and Materials

In this experiment twenty sterile Petri plates were poured with 2% Difco Agar (Water Agar = WA). Because of its nutritionless quality, WA was used to avoid promoting rapid mycelial growth of the test fungus and to prevent the growth of any possible contaminants in the nematode inoculum. When the medium had solidified 7 mm discs were removed as in experiment one above. Inserted into the spaces remaining were discs of fungi cultured on PDA. In addition to the six fungi used in the previous experiment, the following fungi were tested for attractiveness to A. avenae: Verticillium cinnabarinum, Berk.; S. bataticola, Gliocladium roseum, Bainier; Aspergillus niger, van Tiegh.; Trichothecium roseum, Link; and Helminthosporium sativum Pamm., King and Bakke. The fungi were arranged in groups of three as follows: Group A: T. viride, S. sclerotiorum

and Fusarium sp.; Group B: V. albo-atrum, V. dahliae and V. cinnabarinum; Group C: F. culmorum, S. bataticola and G. roseum; Group D: A. niger, T. roseum and H. sativum; Group E: Control, no fungi.

Preparation of A. avenae inoculum was the same as in experiment one, except that the nematodes were not treated with $HgCl_2$ and instead were washed four times with sterile distilled water. The nematodes were concentrated to 3.0 ml and 0.1 ml aliquots were pipetted to the center of each fungal inoculated plate. Four replicates were used for each treatment. Control plates contained discs of PDA only. The nematodes were added immediately after the different fungi were inoculated. The immediacy of nematode addition in relation to fungal inoculation was made necessary in order to avoid extensive fungal growth toward the nematode inoculation zone.

The plates were incubated in the dark at 24°C and at 18 and 24 hours, the number of nematodes within each fungal colony was determined using a stereomicroscope.

(ii) Results

The results of this experiment are summarized in Tables 13, 14 and Figure 6. Because A. avenae frequently fed on submerged hyphae of different fungi, it was often necessary to invert the plate in order to count the number of nematodes within a fungal zone.

At 18 hours after inoculation, there were widespread differences (not significant statistically, Appendix Table V) in the number of A. avenae counted in different fungus colonies (Table 13). A. avenae was attracted most strongly to Fusarium sp. (average 14.2) and V. dahliae (9.7). Several fungi (G. roseum (5.7), T. roseum (3.7), T. viride (3.2), V. cinnabarinum (3.2), A. niger (3.2) and F. culmorum (2.0) appeared to be only slightly attractive to A. avenae at this time. The number of nematodes observed in colonies of S. sclerotiorum, V. albo-atrum, S. bataticola and H. sativum were equal to or less than the number in control PDA discs, indicating that these fungi were unattractive to A. avenae.

From the data summarized in Table 14, it can be seen that after an incubation of 24 hours, the greatest number of nematodes were found in colonies of F. culmorum (average 22.0) followed closely by V. albo-atrum (21.5) and V. cinnabarinum (20.5). Large numbers were also found in Fusarium sp. (19.0), T. viride (18.2) and G. roseum (17.2). A. avenae was also attracted to T. roseum (15.0) and V. dahliae (14.5). Several fungi in this experiment were unattractive to A. avenae. The number of nematodes in colonies of S. sclerotiorum (2.2), S. bataticola (1.5) and H. sativum (2.2) closely resembled the number found in the control discs of PDA. In the majority of cases, nematodes found within a fungal colony were feeding or probing on the mycelial strands. None of the above differences between treatment means were significantly different, however (Appendix Table VI).

Table 13. Preferential selection of different soil fungi by A. avenae in a WA medium

Fungus Grouping	No.[2] of A. avenae Per Replicate in Fungus or Control Zone					
	1	2	3	4	Total	Average
Group A						
T. viride	4	2	3	4	13	3.2[a1]
S. sclerotiorum	0	0	0	0	0	0[a]
Fusarium sp.	2	17	26	12	57	14.2[a]
Group B						
V. albo-atrum	0	0	0	3	3	0.7[a]
V. dahliae	6	28	5	0	39	9.7[a]
V. cinnabarinum	1	7	0	5	13	3.2[a]
Group C						
F. culmorum	1	5	1	1	8	2.0[a]
S. bataticola	1	0	0	1	2	0.5[a]
G. roseum	5	4	2	12	23	5.7[a]
Group D						
A. niger	0	6	0	7	13	3.2[a]
T. roseum	0	13	0	2	15	3.7[a]
H. sativum	0	0	0	0	0	0[a]
Group E						
Control	1	0	2	0	3	0.7[a]

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]Determined 18 hours after inoculation.

The number of A. avenae entering certain fungal colonies increased sharply between 18 hours and 24 hours (Figure 6). This can be easily determined by subtracting the average number of A. avenae in the 18-hour cultures from the number in the 24-hour cultures. Fungi allowing the greatest increase were V. albo-atrum (20.7), F. culmorum (20.0), V. cinnabarinum (17.2) and T. viride (15.0). Although not as great as the above fungi, G. roseum (11.5) T. roseum (11.2) and A. niger (7.5) demonstrated fairly marked increases. The values for V. dahliae (4.7) and Fusarium sp. (4.7) were low, but it is interesting to note that both fungi had the highest number of A. avenae of all the fungi in the 18-hour test. Three fungi, H. sativum (2.2) S. sclerotiorum (2.2) and S. bataticola (1.0), demonstrated very slight increases in the number of nematodes entering their colonies between 18 and 24 hours.

c. Experiment Three

The previous two experiments indicated that A. avenae may preferentially select certain fungi when given a choice on agar media. Since soil is the natural medium for A. avenae, it was decided to determine whether A. avenae would select specific fungi in a soil medium.

(i) Methods and Materials

In order to have adequate comparison, the same fungi and grouping arrangements were used in this experiment as used in experiment two. The method used to culture the different fungi on soil was similar to that of Marshall and Alexander (1960).

Table 14. Preferential selection of different soil fungi by A. avenae in a WA medium

Fungus Grouping	No.[2] of A. avenae Per Replicate in Fungus or Control Zone 1	2	3	4	Total	Average
Group A						
T. viride	10	27	8	28	73	18.2[a][1]
S. sclerotiorum	2	2	2	3	9	2.2[a]
Fusarium sp.	4	17	31	24	76	19.0[a]
Group B						
V. albo-atrum	17	14	44	11	86	21.5[a]
V. dahliae	10	34	12	2	58	14.5[a]
V. cinnabarinum	5	19	35	23	82	20.5[a]
Group C						
F. culmorum	35	19	22	12	88	22.0[a]
S. bataticola	3	1	0	2	6	1.5[a]
G. roseum	19	9	5	36	69	17.2[a]
Group D						
A. niger	6	17	10	10	43	10.7[a]
T. roseum	3	34	10	13	60	15.0[a]
H. sativum	7	1	1	0	9	2.2[a]
Group E						
Control	4	7	4	3	18	4.5[a]

[1] Any two means in a column with the same superscript are not significantly different. ($P \leq 0.05$).

[2] Determined 24 hours after inoculation.

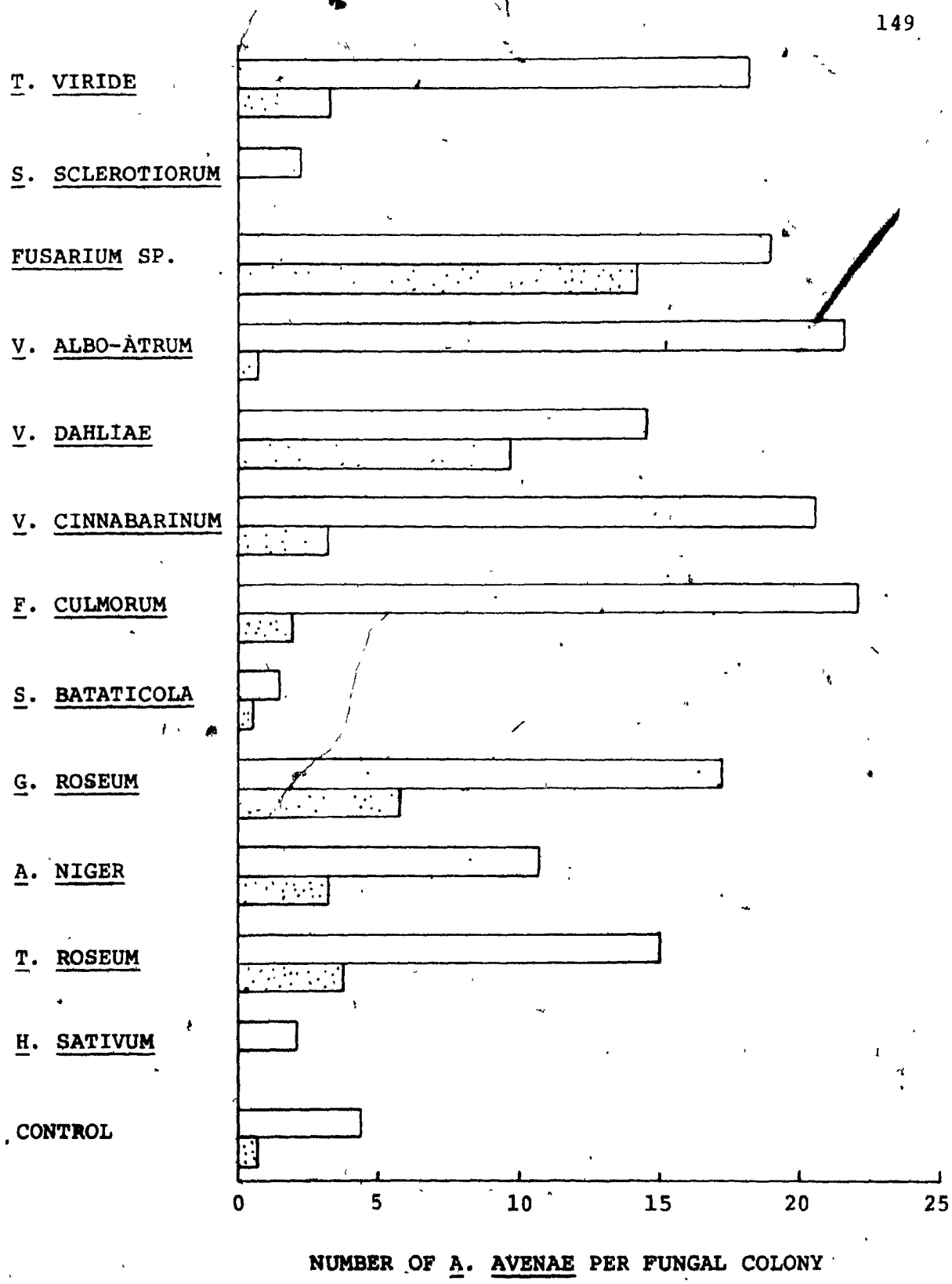


Figure 6. Preferential selection by A. avenae of different soil fungi. 18 hours 24 hours

Each of twenty clean Petri dishes were given 35 g of an air-dry sandy loam soil enriched with glucose. Glucose was added to the soil to give a sugar-soil ratio of 0.5% on a weight/weight basis. This was accomplished by mixing carefully 5 g of glucose with 1,000 g of the above air-dry soil. The soil moisture content was adjusted to 60% of the water-holding capacity of the soil. This value is close to the upper limit for optimal microbial activity in soil (Alexander, 1961). The plates were left for 12 hrs for the moisture to equilibrate evenly throughout the soil. They were then autoclaved at 101.3 kPa for 20 minutes. After cooling, three 7 mm soil cores were removed from each plate at equal distances from the center of the plate and equidistant from each other. A sterile 7 mm cork borer was used to remove the soil cores.

Each vacant space left by removal of the soil core was given a 7 mm disc of PDA cultured fungus. The fungus inoculated plates were left at room temperature for 24 hrs. Control plates received sterile discs of PDA.

Nematode inoculum was prepared as in experiment one. Aliquots of 0.2 ml were placed in the center of each soil plate. The number of nematodes in each 0.2 ml volume varied from 50 to 80. The plates, four per treatment, were incubated at 24°C in the dark for 48 hours.

Upon termination of the 48 hour incubation period, each disc of fungus or agar plus surrounding soil to a diameter of 3.5 cm was removed using a 3.5 cm diameter cork borer. The soil and agar discs within each 3.5 cm zone were transferred to

Baermann funnels and nematode counts were made 24 hours later.

(ii) Results

The data of this experiment is presented in Table 15 and Figure 7.

After a period of 48 hr, only T. viride, with an average of 33.0 nematodes per colony was significantly preferred (Appendix Table VII) by A. avenae, compared with control PDA discs. Large but not significant numbers of A. avenae were also found in the fungal-soil zones of V. albo-atrum (29.7) and G. roseum (27.5). Three other fungi recorded high averages; Fusarium sp. (23.2), A. niger (23.0) and F. culmorum (21.5). Very few nematodes could be found in the fungal-soil zones of S. sclerotiorum (10.0), T. roseum (7.0), V. cinnabarinum (5.5), H. sativum (5.5) and S. bataticola (4.5).

Despite differences in incubation time and type of medium, similar responses by A. avenae to most fungi can be observed in experiments two and three. Three fungi gave different results, however. More nematodes were attracted to S. sclerotiorum in the soil medium than on agar whereas the reverse was true for V. cinnabarinum and T. roseum.

Table 15. Preferential selection of different soil fungi by A. avenae in a glucose amended soil medium

Fungus Grouping	No. of A. avenae Per Replicate in Fungus or Control Zone 1	2	3	4	Total	Average
Group A						
T. viride	25	27	44	36	132	33.0[a][1]
S. sclerotiorum	22	3	6	9	40	10.0[ab]
Fusarium sp.	17	29	28	19	93	23.2[ab]
Group B						
V. albo-atrum	47	26	8	38	119	29.7[ab]
V. dahliae	21	17	19	12	69	17.2[ab]
V. cinnabarinum	5	8	6	3	22	5.5[b]
Group C						
F. culmorum	36	23	12	15	86	21.5[ab]
S. bataticola	1	5	10	2	18	4.5[b]
G. roseum	19	23	39	29	110	27.5[ab]
Group D						
A. niger	15	24	33	20	92	23.0[ab]
T. roseum	5	9	8	6	28	7.0[ab]
H. sativum	6	5	10	1	22	5.5[b]
Group E						
Control	10	3	11	0	24	6.0[b]

[1]Any two means in a column with the same superscript are not significantly different (P≤0.05).

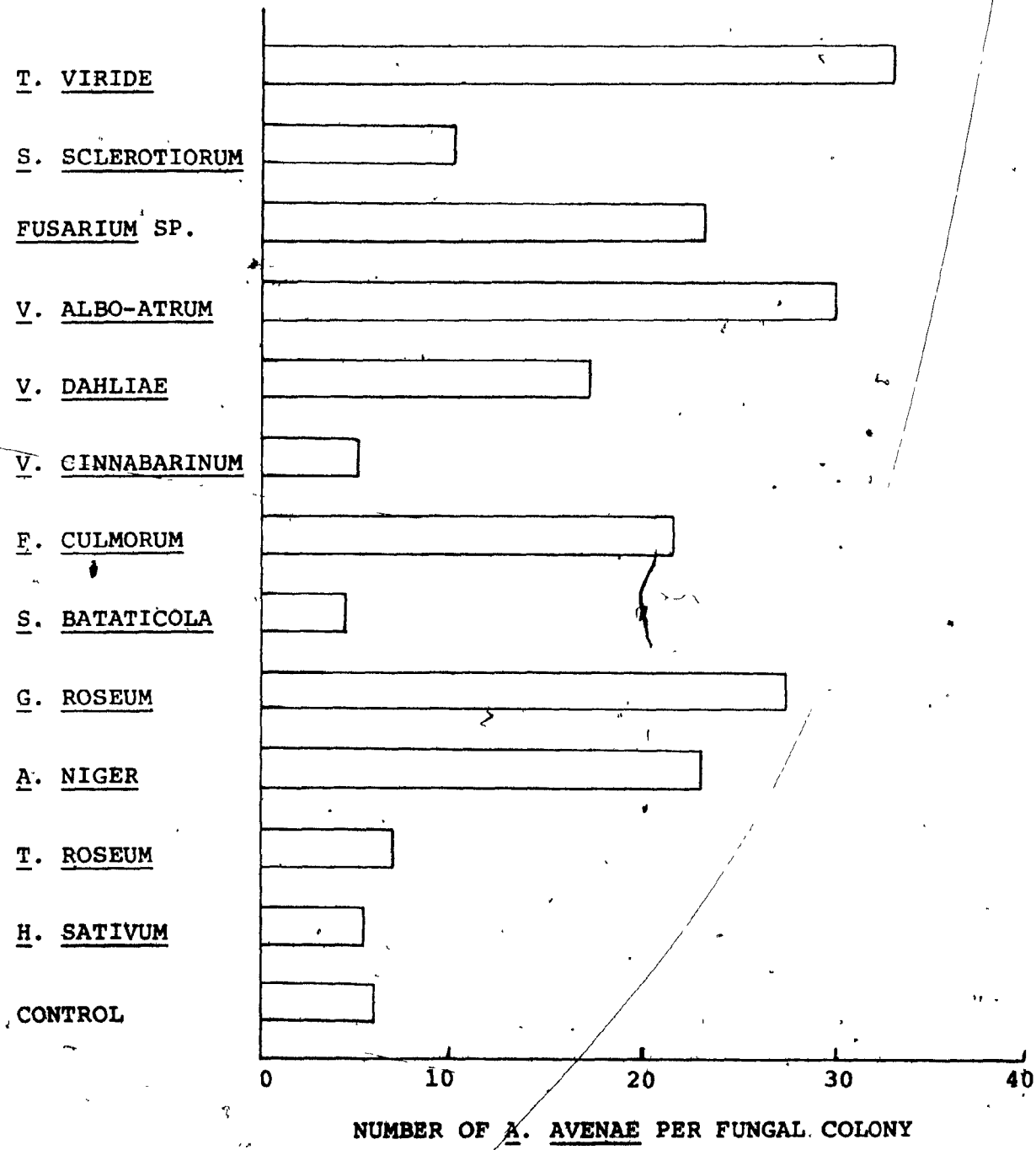


Figure 7. Preferential selection by A. avenae of different fungi cultured in soil.

2. Growth and Multiplication of A. avenae on Fungi Cultured in a Soil Medium

The potential of different fungi to support multiplication of A. avenae and other mycophagous nematodes has received considerable attention in recent years. Mankau and Mankau (1962) noted that A. avenae multiplied in large numbers on phytopathogenic fungi, in smaller numbers on saprophytic soil fungi and not at all on several Pythium and Phytophthora species. Isolates of R. solani were found to vary greatly in their capacity to support large populations of A. avenae (Barker, 1964). A study of the fungus hosts of A. avenae by Townshend (1964) revealed that 54 of the 59 species that he used were hosts. The influence of ten fungi on host preference, host suitability and morphometrics of five mycophagous nematodes were studied by Pillai and Taylor (1967). For A. avenae, these authors rated the fungi as follows: six fungi were excellent hosts, one was good and three were non-hosts. Evans and Fisher (1970b) found that A. avenae reproduced on seven of eight fungi tested.

It must be noted that the above research on fungus host efficiency for A. avenae and certain other mycophagous nematodes has been completed using nutrient-supplemented agar media. To date, no work has been done using soil, the natural habitat for A. avenae, and the fungi it feeds on. Also, the effect of time on the growth of A. avenae on different fungi has not been

determined. In this section of research the objective was, therefore, to determine the fecundity of A, avenae on various soil-borne fungi using a soil system.

a. Experiment One

A preliminary experiment had shown that certain soil-borne fungi could grow on steamed soil under well-defined laboratory conditions. In this experiment, the ability of A. avenae to feed and multiply on soil-grown fungi was determined.

(i) Methods and Materials

Glucose amended sandy loam soil was prepared by adding glucose to dry soil so as to give glucose/soil concentrations of 0, 1 and 4% glucose on a weight/weight basis. Ten grams of the amended dry soil were placed in each of fourteen 60 x 15 mm Petri dishes and, using distilled water, the soil in each dish was adjusted to 40% of the moisture-holding capacity. The plates were autoclaved at 101.3 kPa for 20 min. When cool, each plate was given 1 ml of a mycelial-spore suspension of either Fusarium sp. or T. viride. There were two replicates per treatment. Addition of the inoculum was calculated to adjust the previously moistened soil to 50% of its moisture-holding capacity. The cultures were incubated in the dark at 24°C for 14 days. The plates were placed in polyethylene bags to ensure minimal moisture loss during incubation. After 14 days, approximately 100 nematodes were added to each culture.

These were from a suspension of A. avenae that had been carefully washed 5 times in sterile distilled water, after 0.01% $HgCl_2$ treatment, and adjusted so that the concentration of nematodes was 100 per ml. After A. avenae inoculation, all cultures were incubated at 24°C for 14 days after which the content of each dish was transferred to a Baermann funnel, left for 20 hr and the number of nematodes determined in a counting dish. Because the population of A. avenae in certain treatments was very large, suitable dilutions were made before counting was undertaken. The data were not analyzed statistically due to the preliminary nature of this experiment.

(ii) Results

From the data presented in Table 16 and Figure 8, it is readily apparent that development and reproduction of A. avenae is supported by soil-borne fungi. In this experiment, greater numbers of A. avenae were produced on the Fusarium sp. than on T. viride. The effect of the glucose amendment on nematode numbers is clearly revealed in Fig. 8. The progressive increase in nematode numbers with glucose concentration is probably due to the corresponding increase in fungus mycelial growth. At the time of nematode addition the growth of Fusarium sp. on the 1% and 4% glucose amended soils had covered the entire soil surface, appearing as a dense, cottony mass. Growth by this fungus on unamended soil was extensive enough to cover the soil surface but it appeared very sparse. T. viride showed a

similar pattern, with light mycelial coverage on the unamended soil and a very dense, heavily sporulating coverage of the 1% and 4% glucose amended soil.

Table 16. Effect of fungi, cultured on glucose amended soil, on the number of A. avenae

Concentration of Glucose	Replicate	Number[a] of A. Avenae Per Plate		
		Fusarium sp.	T. viride	Control
0%	1	600	50	10
	2	500	80	30
1%	1	2,140	220	-[b]
	2	2,560	300	-[b]
4%	1	3,070	960	-[b]
	2	4,330	1,260	-[b]

[a]Nematodes were harvested 14 days after inoculation.

[b]Glucose amended controls were not used.

b. Experiment Two

In view of the results of the previous experiment, where it was shown that two soil-grown fungi were capable of supporting extensive multiplication of A. avenae, it was decided to extend the number of test fungi in an additional experiment. Because of a report in the literature (Katznelson and Henderson, 1964) indicating that certain cultures of soil actinomycetes

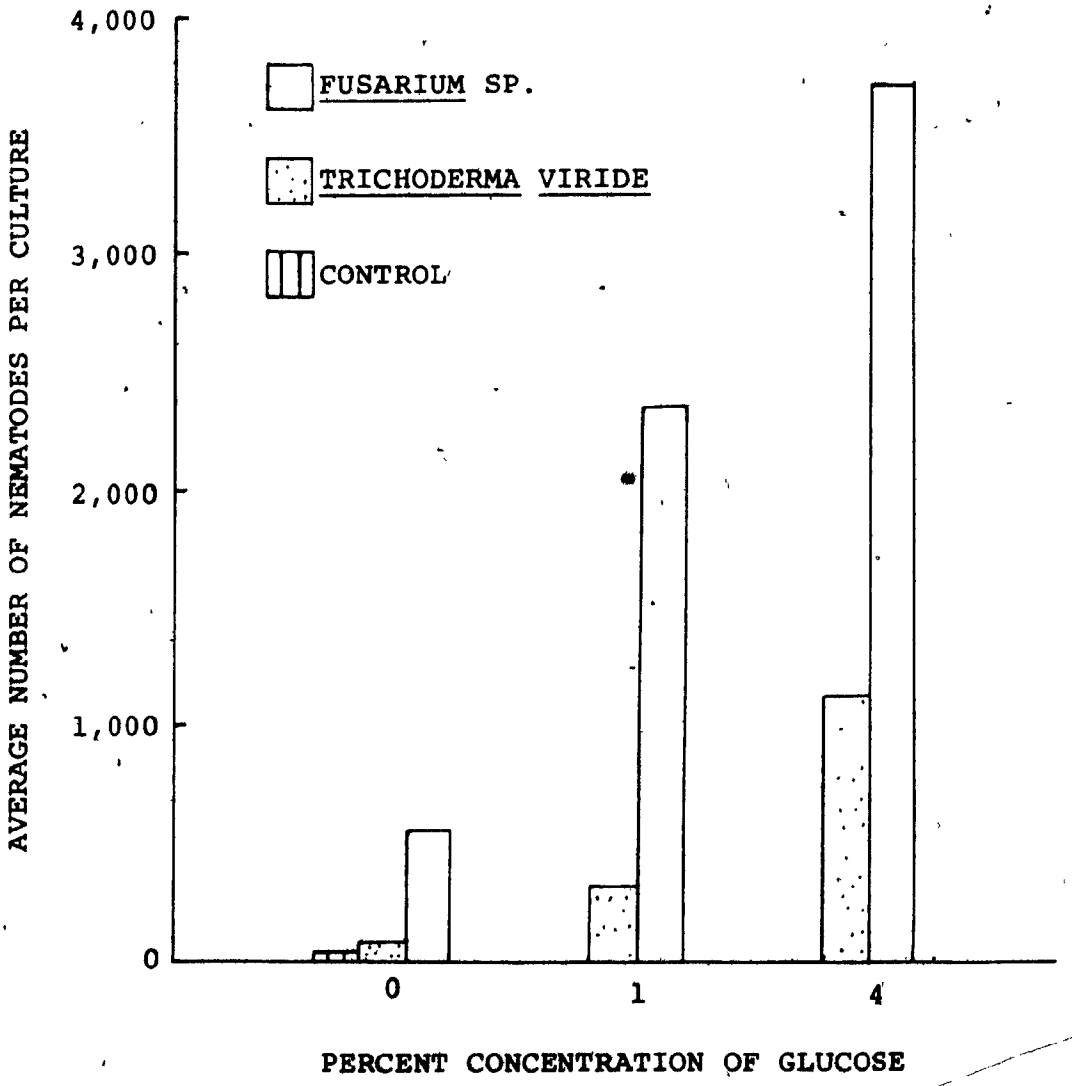


Figure 8. Growth of A. avenae on two soil fungi cultured in glucose amended soil.

were highly attractive to Aphelenchoides parietinus, two isolates of soil actinomycetes were also included.

(i) Methods and Materials

The following fungi were used in this experiment: Fusarium sp.; Trichoderma viride; Sclerotium bataticola; unidentified species of Verticillium, Nees and Pullularia, Berk; and two different but unidentified isolates of actinomycetes. All fungi except Pullularia sp. were maintained on PDA. The Pullularia sp. was grown on a yeast-extract supplemented malt agar medium. The actinomycete isolates were isolated from soil and maintained on a medium recommended by Kuster and Williams (1964).

Ten grams of an air dry sandy loam soil, previously screened through a number 10 mesh sieve (USS) and amended with 0.5% glucose, were placed into each of ninety-six 60 mm x 15 mm Petri dishes. These units were autoclaved at 101.3 kPa for 20 min. The following day, 70 ml mycelial-spore suspensions of the fungi and actinomycetes, except for Verticillium, were prepared by carefully homogenizing, in a sterile blender, scrapings from the surface of each colony. Using sterile 5 ml pipettes, 3.5 ml of the above homogenates were pipetted onto the surface of the previously autoclaved soil. This amount was calculated to bring the moisture content of the soil to 58% of its moisture-holding capacity. The culture of Verticillium was cut into small plugs, using a 4 mm sterile cork borer, and transferred to the appropriate plate of sterile soil. Each treatment was replicated three times. The cultures were

incubated in the dark at 24°C for 6 days.

A. avenae nematode inoculum was obtained from cultures grown on S. bataticola. Collection and surface sterilization techniques were the same as in the previous experiment. One ml aliquots, containing approximately 150 nematodes, were distributed near the center of each plate. At the time of nematode inoculation several of the fungi had grown over a large proportion of the soil surface. Mycelia of T. viride occupied the entire surface and was rapidly forming spores. S. bataticola and Fusarium sp. had completely covered the soil surface with large amounts of aerial mycelia. Colonies of Verticillium sp. were only 1 cm in diameter and consisted of dense mycelial growth. There was no visible evidence of growth by the Pullularia sp. Both actinomycete cultures had formed dense localized areas of conidial growth where the pipetted inoculum had contacted the soil. It was apparent that these colonies were not spreading. Control plates remained free of microorganisms. All nematode inoculated cultures were incubated in the dark at 24°C.

Nematode counts were made at 4, 8, 12 and 16 days. Both adults and juveniles were counted. Procedures of nematode extraction and counting were the same as in the previous experiment.

(ii) Results

A summary of the results of this experiment are presented in Table 17 and Figure 9.

Table 17. The effect of different fungi and actinomycetes on the fecundity of A. avenae

Organism	Number[2] of A. avenae Extracted, Time in Days 4	8	12	16
S. bataticola	510.0[a1]	4,650.0[a1]	4,186.6[a1]	3,036.6[a1]
Fusarium sp.	341.6[abc]	2,315.0[b]	2,040.1[b]	1,713.3[ab]
T. viride	421.6[ab]	2,130.0[b]	1,450.0[b]	633.3[bc]
Verticillium sp.	175.0[bcd]	1,833.3[b]	973.3[b]	406.6[c]
Pullularia sp.	96.6[d]	98.3[c]	106.6[c]	35.0[c]
Actinomycete a	153.3[cd]	100.0[c]	33.3[c]	38.3[c]
Actinomycete b	128.3[cd]	81.6[c]	53.3[c]	48.3[c]
Control	61.6[d]	115.0[c]	51.6[c]	33.3[c]

[1]Any two means in a column with the same superscript are not significantly different (P≤0.05).

[2]Average of three replicates.

From the data presented in Table 17 and graphed in Figure 9, it is clear that the fungi tested varied greatly in ability to support A. avenae. Multiplication of A. avenae, compared with the control, was significantly greater (Appendix Table VIII), at 4 days, on S. bataticola, Fusarium sp. and T. viride but not on Verticillium sp., Pullularia sp. and both actinomycete isolates. At 8 days, nematode numbers were significantly

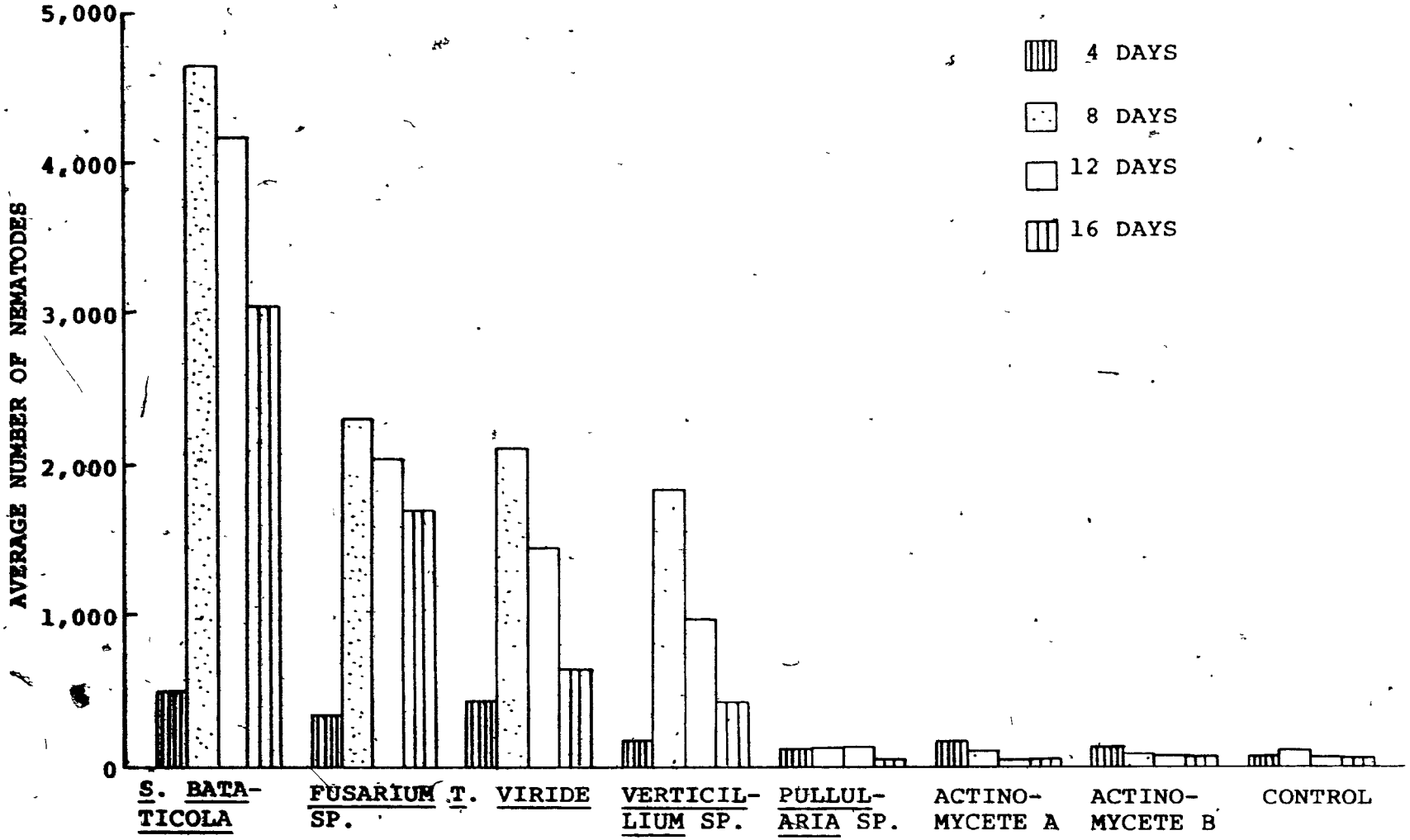


Figure 9. Multiplication of A. avenae on several soil fungi and actinomycetes.

greater (Appendix Table IX) on S. bataticola, Fusarium sp., T. viride and Verticillium sp. than on the control, with the population of A. avenae on S. bataticola being significantly larger than on the other three fungi. The same pattern, which was observed at 8 days, occurred also at 12 days, with significantly greater numbers (Appendix Table X) of A. avenae occurring on S. bataticola compared with Fusarium sp., T. viride and Verticillium sp. Sixteen days after nematode inoculation only S. bataticola and Fusarium sp. supported significantly larger (Appendix Table XI) populations of A. avenae compared with the control. Although average values for nematode numbers on T. viride and Verticillium sp. appeared much greater than the control, the differences were not significant statistically.

Nematode multiplication, due to egg laying of the gravid females, was slow during the first 4 days. Between 4 and 8 days of incubation, numbers of A. avenae increased rapidly, reflecting the maturation and reproduction of the juveniles from the previously laid eggs. For various reasons, the populations of A. avenae supported by the different fungi declined after 8 days and appeared to be still declining at the end of the experiment (16 days). One explanation for the decline is that after 8 days, and with limited nutrient supply, the fungus mycelium available for nourishing the rapidly increasing nematode population, began to diminish. It is also possible that there was a build-up of substances which were toxic to the

nematodes or inhibited hatching of their eggs.

From the data in Table 17, it can be seen that the number of nematodes recovered from the Pullularia sp. and the two actinomycete isolates closely resembled those of the control, indicating that A. avenae was unable to reproduce on these organisms.

c. Experiment Three

The above experiment was repeated with modifications as noted below, and extended to include Sclerotinia sclerotiorum, an identified species of Fusarium and two of Verticillium.

(i) Methods and Materials

Fungi used in this study were isolated from soil or diseased plant material and maintained on PDA. They included T. viride; S. sclerotiorum; Fusarium sp.; F. culmorum; V. albo-atrum and V. dahliae.

Eighty-four 60 mm x 15 mm Petri dishes were given ten grams of a glucose amended soil as used before.

The soil was adjusted to 58% of the moisture holding capacity with the addition of 3.5 ml of distilled water. The plates were left overnight for the water to diffuse evenly throughout the soil. The uniformly moist plates were then autoclaved for 20 min at 101.3 kPa the following day. Upon cooling to room temperature, the plates were inoculated with

4 mm discs of fungi cut with a sterile cork borer. The culture dishes were wrapped in plastic bags to prevent moisture loss and incubated in the dark at 25°C for a period of 7 days. Fungal growth, with the exception of both Verticillium sp. and S. sclerotiorum, consisted of extensive mycelial coverage of the soil surface by the seventh day. The color of the plates inoculated with T. viride was dark green, indicative of heavy spore formation. Plates of S. sclerotiorum were three quarters covered by a cottony aerial growth, with tufts of mycelia irregularly spaced over the soil surface. These mycelial tufts probably represented the initial stages of sclerotia formation. Growth of V. albo-atrum and V. dahliae was slight, with dense white mycelial growth covering only one quarter of the plate.

Approximately 42 adult A. avenae nematodes, in 1 ml aliquots, were added to the center of each plate culture after 7 days of fungus growth. Preparation of the nematode inoculum was the same as in the previous experiment. Details of incubation, number of replicates, times of harvest and method of determining nematode numbers were the same as in the above experiment.

(ii) Results

A summarization of the results of this experiment is found in Table 18 and Figure 10.

As in the previous experiment, the fungi tested were extremely variable in their ability to support feeding and

Table 18. The effect of different soil-borne fungi on multiplication of A. avenae

Fungus	Number[2] of A. avenae Extracted Time in Days 4	8	12	16
F. culmorum	226.6^{ab}[1]	3,016.6^{a}[1]	22,470.0^{a}[1]	22,370.0^{a}[1]
Fusarium sp.	241.6^{a}	1,043.3^{b}	1,531.6^{b}	1,370.0^{b}
V. albo-atrum	168.3^{ab}	700.0^{b}	1,156.6^{b}	1,320.0^{b}
S. sclerotiorum	186.6^{ab}	910.0^{b}	806.6^{b}	225.0^{c}
T. viride	155.0^{ab}	680.0^{b}	540.0^{b}	446.6^{bc}
V. dahliae	128.3^{ab}	725.0^{b}	428.3^{b}	225.0^{c}
Control (No Fungus)	16.6^{b}	18.3^{b}	40.0^{b}	8.3^{c}

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]Average of three replicates.

multiplication of A. avenae. Compared with the control, numbers of A. avenae at 4 days were significantly greater (Appendix Table XII) on only one fungus, namely Fusarium sp. Despite the fact that average values for nematode numbers on the remaining five fungi were much greater than the control, the differences were not statistically significant. At 8 days, nematode numbers were significantly greater (Appendix Table XIII) on F. culmorum but not on Fusarium sp., V. albo-atrum, S. sclerotiorum, T. viride and V. dahliae. The same results were also observed at 12 days (Appendix Table XIV). Sixteen days after nematode

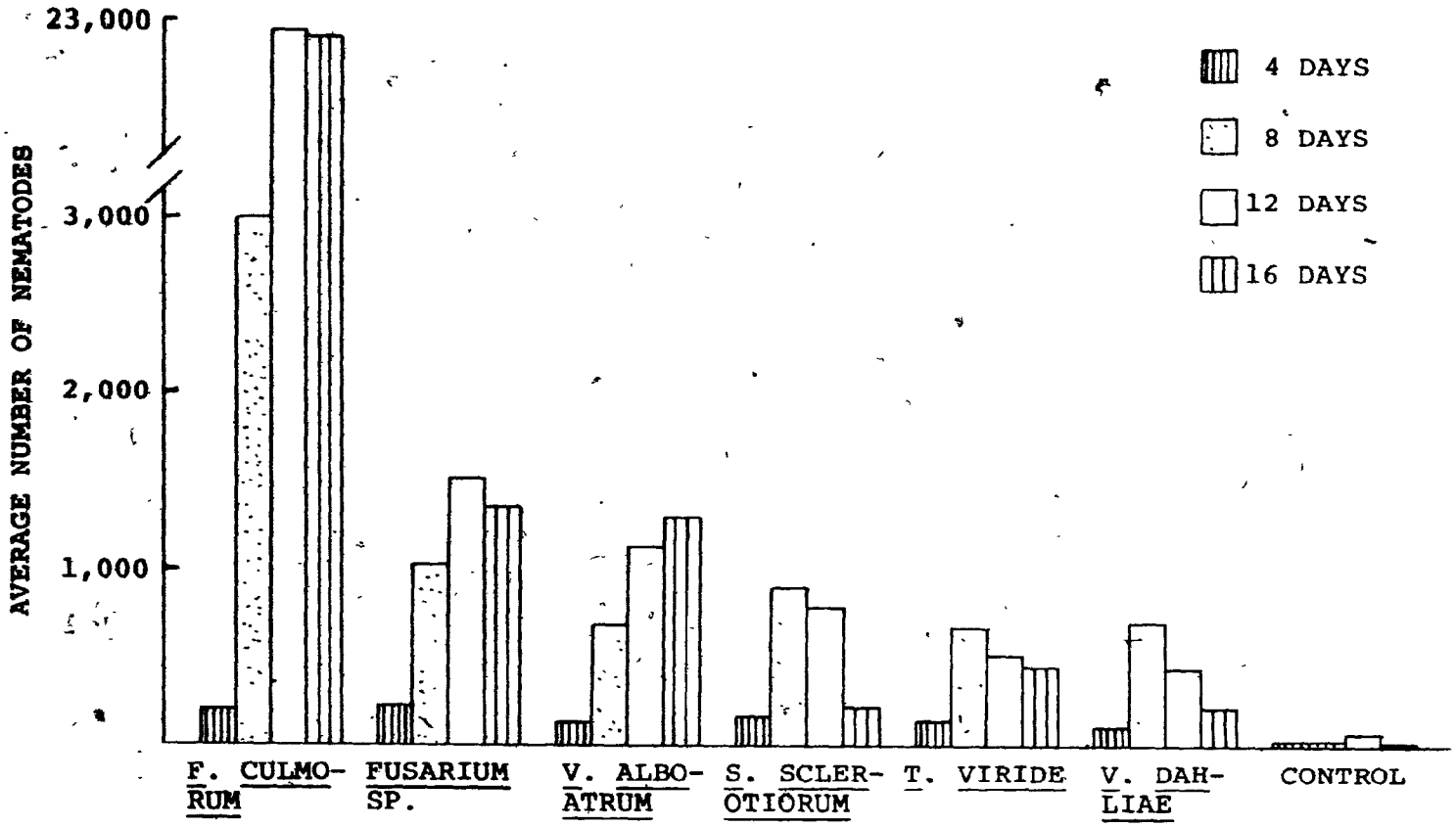


Figure 10. Multiplication of A. avenae on different soil fungi.

inoculation, F. culmorum, Fusarium sp. and V. albo-atrum supported significantly greater (Appendix Table XV) progeny of A. avenae compared with the control. At this time, multiplication of A. avenae on F. culmorum was statistically greater compared with Fusarium sp. and V. albo-atrum.

The maximum number of nematodes was reached in 8 days by S. sclerotiorum, T. viride and V. dahliae, whereas F. culmorum and Fusarium sp. required 12 days for total A. avenae population development. One fungus, namely V. albo-atrum, supported continuous population development of A. avenae whose numbers were still increasing at 16 days. The most rapid rate of A. avenae multiplication occurred on F. culmorum. Nematode numbers declined rapidly after 8 days in cultures of S. sclerotiorum, T. viride and V. dahliae, whereas only slight reductions in numbers of A. avenae occurred in both Fusarium cultures after 12 days.

3. Effect of A. avenae on Nodulation of Bean Roots by Rhizobium phaseoli

Investigation into the role of certain nematodes in the pathology and aetiology of plant root lesions convinced Goodey (1935) that A. avenae was not a plant parasite, but instead, was closely associated with moribund root tissue. A year later, Steiner (1936) vigorously refuted Goodey's claim and presented histological evidence that A. avenae not only could migrate

through root (phlox hybrid) parenchyma tissue but was capable of reproduction within the root system. Since Goodey and Steiner's early work on the status of A. avenae as a plant parasite, evidence has accumulated showing that A. avenae can feed on root hairs of several higher plants (Chin and Estey, 1966), penetrate the roots of corn and tomato (Terry, 1966), and reproduce on certain tissue cultures of higher plants (Barker, 1963; Chin, 1964; Barker and Darling, 1965). Not all work has showed that A. avenae could parasitize higher plant roots. Sutherland (1967) and Sutherland and Fortin (1968) failed to find A. avenae in the roots of seven species of conifer seedlings. They concluded that A. avenae could not enter or feed upon intact, healthy tissues of higher plants.

In view of the somewhat controversial situation concerning parasitism of higher plants by A. avenae and the ubiquity of this nematode in the rhizosphere of plants, especially legumes, it was decided to determine whether A. avenae would affect the nodule forming process of beans by R. phaseoli.

a. Experiment One

This experiment was designed to determine whether A. avenae would affect nodulation of beans by R. phaseoli. An excised root technique was used.

(i) Methods and Materials

Excised roots of a Pencil Pod cultivar of beans Phaseolus

vulgaris, were the test "plants" of this experiment. Techniques used in seed germination, root excisement, preparation of inorganic and organic media, and culture vessel sterilization were the same as in previous experiments described in Part I, Section 3.

Fresh R. phaseoli inoculum was prepared by transferring ten loopfuls of mucoid bacterial growth, taken from 48 hr Congo Red Mannitol Agar (CRMA) slant cultures, to 50 ml of sterile distilled water in a sterile, rubber-stoppered 125 ml Erlenmeyer flask. The contents were vigorously shaken and 1 ml aliquots were distributed to appropriate excised roots.

Large numbers of A. avenae, previously cultured on S. bataticola, were collected in Baermann funnels. The nematodes were surface sterilized for 3 min in 0.01% $HgCl_2$ and washed three times in sterile tap water. Concentrations of 100/ml, 1,000/ml and 10,000/ml were prepared using suitable dilutions. One ml volumes of each concentration were added to the appropriate treatment. To test for efficiency of surface sterilization, 0.1 ml aliquots of each nematode concentration were pipetted to plates of sterile Difco Nutrient Agar. After an incubation time of 48 hr at 24°C, the plates were examined using a stereomicroscope. Surface sterilization was judged successful since nematode activity was not impaired and all microorganisms were eliminated.

The treatments, each replicated five times, were as follows:

1. Control, bean roots only
2. R. phaseoli
3. 100 A. avenae
4. 1,000 A. avenae
5. 10,000 A. avenae
6. R. phaseoli + 100 A. avenae
7. R. phaseoli + 1,000 A. avenae
8. R. phaseoli + 10,000 A. avenae

All treatments were randomized and transferred to a dark, ventilated incubator at 24°C. The experiment was terminated after 24 days. The root system in each culture vessel was removed carefully and its fresh weight and number of nodules determined.

(ii) Results

A summarization of the results of this experiment is presented in Table 19.

Upon incubation of the inoculated units, several excised roots were observed to be slightly above the surface of the moist silica sand. However, after an incubation period of 24 hr, these aerial roots were bending toward the surface and had penetrated it by 96 hr. Nodule formation was first observed through the transparent wall of the culture vessel on the ninth day.

Table 19. Effect of different numbers of A. avenae on weight and nodulation of excised bean roots

Treatment	Fresh Weight of Roots (g)[2]	No. of Nodules Per Root[2]	No. of Nodules /g Root Fr.Wt.[2]
Control	0.360	0[a1]	0[b1]
R. phaseoli	0.348	16.2[a]	47.18[a]
100 A. avenae	0.403	0[a]	0[b]
1,000 A. avenae	0.416	0[a]	0[b]
10,000 A. avenae	0.380	0[a]	0[b]
R. phaseoli + 100 A. avenae	0.362	14.6[a]	38.60[a]
R. phaseoli + 1,000 A. avenae	0.387	15.4[a]	39.61[a]
R. phaseoli + 10,000 A. avenae	0.386	12.0[a]	30.19[ab]

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]Average of five replicates.

From Table 19 it can be seen that fewer nodules formed on bean roots exposed to all concentrations of nematode inoculum. However, differences between treatment means were not significant, both for nodules per root (Appendix Table XVI) and nodules per gram of root (Appendix Table XVII). The fewest nodules occurred in treatments containing 10,000 A. avenae, where nodulation was 25.9% less than in the treatments with R. phaseoli alone.

The other two inoculum levels of A. avenae inhibited nodule formation to a lesser degree. With regard to fresh weight of the root systems, although initial excised root weight was not measured, there were only slight differences between replicates and between treatments.

b. Experiment Two

In the above experiment, A. avenae prevented nodulation on excised roots of Phaseolus vulgaris, cultivar Pencil Pod. In order to observe whether this nematode could feed on roots of beans, a preliminary investigation was carried out using an agar medium for bean root culture in Petri dishes.

(i) Methods and Materials

Preparation of bean seeds for germination, and later, for root excisement, was the same as described in the above experiment.

Because of heavy precipitation occurring when calcium, sulphate and phosphate compounds were autoclaved together, it was necessary to modify the composition and method of preparation of Cartwright's (1967) inorganic medium for excised root culture. Observation of nematode movement around excised roots was greatly improved by substituting $CaCl_2 \cdot 6H_2O$ for $CaCO_3$. Five hundred ml of modified Cartwright's medium was prepared as follows: 223 mg of $CaCl_2 \cdot 6H_2O$ and 100 mg of KH_2PO_4 were dissolved separately in 50 ml of distilled water and sterilized

by autoclaving. When cool, each 50 ml volume was divided in half, giving two 25 ml volumes of calcium and two 25 ml volumes of phosphate. Using a magnetic stirrer, all other chemicals were dissolved in 400 ml of distilled water in a 500 ml Erlenmeyer flask. When the solution was clear, 200 ml were removed and transferred to another 500 ml Erlenmeyer flask. To each 200 ml volume was added 3.75 g of Difco Agar, the contents blended well and autoclaved at 101.3 kPa for 15 min. When the media were cooled to approximately 50°C, each of the previously sterilized 25 ml volumes of calcium and phosphate were added and mixed thoroughly. It was found in previous trials, that very little precipitation occurred when these compounds were mixed at near room temperature, whereas addition at near boiling temperatures resulted in immediate and heavy precipitation. When the media had cooled to approximately 45°C, a 10 ml cell suspension of R. phaseoli was added to one of the media vessels. Depending on the treatment, the warm, inorganic agar medium, plus or minus R. phaseoli cells, was then poured over freshly excised roots contained in 16 sterile Petri dishes. Prior to the addition of the inorganic agar medium, the excised roots were placed into vials of Cartwright's (1967) organic agar medium.

When the medium solidified in the Petri dishes, a 0.1 ml aliquot containing approximately 50 adult A. avenae was added to the surface of the medium in each dish. The cultures were incubated for 3 days in a dark cabinet at 24°C. Egg deposition

and nematode feeding habits on the plant roots were observed periodically using a stereomicroscope.

(ii) Results

Although the length of the experiment was not sufficient for nodule formation, A. avenae was observed to respond more to roots inoculated with R. phaseoli, than to uninoculated roots. In most observations, more nematodes were seen congregating around roots inoculated with R. phaseoli than bacteria-free roots. Several of these nematodes were actively feeding on cells near the root cap area, and, in some instances, were completely immersed in the loose cells of the root cap. R. phaseoli appeared to stimulate A. avenae, as the nematodes moved much more actively and deposited more eggs in the presence of this bacterium than in its absence. Because of the concentration (1.5%) of the agar, nematodes were able to penetrate and migrate easily throughout the agar medium. Actual numbers of nematodes near plant roots were not determined; however, it was generally observed that more nematodes gathered around rootlet tips which had penetrated the agar, than those which were growing along the surface.

c. Experiment Three

In the above preliminary experiment it was demonstrated that in treatments with R. phaseoli, A. avenae accumulated in larger numbers, moved and fed more actively, and deposited more

eggs than they did around roots not inoculated with this bacterium. Therefore, using the same treatments, the objective of this additional experiment was to observe the feeding habits of A. avenae over a much longer period of time than in the above experiment.

(i) Methods and Materials

Procedures used in preparing seeds for germination, root elongation, root excisement and explant culture were the same as in the above experiment.

The method of inoculating the excised roots with R. phaseoli, however, was changed. Roots were dipped in a 10 ml bacterial cell suspension contained in a sterile beaker, and transferred to sterile Petri dishes. Melted and warm inorganic solution agar (Cartwright, 1967) was then poured over the roots of all treatments. The length of roots excised varied from 6 to 12 cm.

Large numbers of A. avenae were cultured on PDA-grown S. bataticola, extracted and surface sterilized in 0.01% $HgCl_2$ for 15 min. After washing four times in sterile tap water, a 0.1 ml aliquot, containing approximately 1,000 nematodes, was pipetted to appropriate treatments. Efficiency of nematode disinfection was tested by transferring 0.1 ml volumes of the treated nematode suspension to plates of Difco Nutrient Agar. After 48 hr, no bacterial or fungal growth was present and nematodes were active, indicating completely efficient nematode surface sterilization.

The treatments, each replicated three times, were as follows:

1. Control
2. R. phaseoli
3. A. avenae
4. R. phaseoli + A. avenae

The cultures were incubated at 24°C in the dark. The experiment was terminated at the eighteenth day. Observations on root growth, nematode behaviour and nodule development were made periodically using a stereomicroscope or compound microscope.

(ii) Results

Uninoculated roots of Phaseolus vulgaris (cultivar, Pencil Pod) grew rapidly, forming numerous lateral roots after 3 days. Many lateral roots penetrated the agar, whereas a few extended along the surface, unable to penetrate. There was little difference in size, after 18 days, between aerial and submerged roots. However, root hair formation by aerial roots was prolific compared with those surrounded by the agar medium. When the experimeht was terminated at 18 days, growth of uninoculated roots was characterized by the formation of numerous, slender, white roots with small areas of discoloration scattered unevenly over the root system.

Despite heavy inoculation with R. phaseoli, excised roots treated with this bacterium did not form nodules. Root morphology, however, was markedly different from the uninoculated

control. After 7 days, bacterial inoculated roots appeared slightly larger than those of the control, and were beginning to attain a brownish color. A thin film of bacterial growth was observed around roots on the surface of the agar. By 11 days, and continuing until the experiment was terminated, there was a noticeable distinction between the size and color of roots that were growing along the agar surface and those that had penetrated. Surface roots were very thick with a somewhat loose, dark layer around them, whereas the submerged roots were much thinner and did not have such a darkened appearance. It should be noted, however, that despite the above difference in size, the submerged inoculated roots were much larger than uninoculated control roots growing on the surface.

The effect of A. avenae on excised root growth was difficult to follow within 24 hr of the experiment because of water films between the roots and surrounding agar. The majority of nematodes were caught in this water film and were extremely difficult to observe under the stereomicroscope. At 3 days, however, the water film disappeared and the nematodes had moved toward rootlets that were penetrating the agar or lying on the surface. The nematodes were actively moving around the rootlets, especially near a root hair, or, in most cases, the root tip. Few nematodes were observed feeding on either root cap cells or root hairs. Feeding was indicated by rapid pulsation of the median oesophageal bulb and movement of food particles from the plant through the oesophagus of the nematode

and into the intestine. A few nematodes could be seen slowly probing root cells and moving from one site along the root to another site, but not inserting their stylets. Complete penetration into the root cap by a few nematodes was observed. Entry was gained by pressing against loose root cap cells, and with slow swaying movement, gradually moving into the root cap. A small number of eggs was seen on the surface of the agar in only one replicate. The majority of the nematodes were mature adults and appeared highly vacuolated. By 7 days, nematodes were still feeding on cells near the root cap. Root hairs formed by submerged roots were severely damaged by the nematodes at the eleventh day. Many of the adults continued to actively feed on cells near the root cap and several continued to probe rootlet surfaces. There was little indication, however, that the nematodes were finding the roots an adequate source of food. This was evident by the short time used for feeding on the root cells; the nematodes would feed for a few seconds, then withdraw their stylet and move on to another site, where the same pattern was repeated. In comparison a suitable fungus host would have been fed upon for several minutes before the nematodes would move to another location. Few eggs were seen, another sign that satisfactory nutrition for reproduction was not being obtained. At 16 and 18 days, the nematodes continued to feed and probe on roots, especially from beneath the agar surface. A small number of juveniles, hatched from previously laid eggs, were observed to be feeding on root parts. Many

adult nematodes appeared almost colorless at this time. The characteristic dark contents in the intestinal and reproductive tracts of rapidly growing A. avenae were absent, a sign of nutritional impoverishment.

The response of A. avenae to roots treated with R. phaseoli was different than it was to axenic roots. In the presence of bacteria, the nematodes were very active, and feeding on root cells was observed often. At 3 days, bacterial growth was visible as a cloudy film around the roots. The nematodes were moving constantly in this zone. Nematode activity appeared to increase by 7 days as many eggs were observed near the tips of submerged roots. Although counts were not made for all roots, clusters of 18, 13 and 35 eggs were found around each of 3 rootlets. These numbers were much higher than any seen around roots in the absence of R. phaseoli. Each rootlet was surrounded by a layer of granular particles, which became disrupted in many places due to nematode activity. By 11 days, many of the eggs laid previously had hatched and the adults, after much movement and feeding on the submerged roots, had moved away from the damaged roots. In several instances the adults were observed to be moving around the roots, but at a distance, and not attempting to feed. In two replicates, small nodules were seen to be forming on aerial rootlets. Not all rootlets were damaged by the nematodes. At 16 and 18 days, several nodules had formed on the aerial portions of some roots. Severe damage of many submerged rootlets was evident. By this time the nematodes had ceased to feed on the damaged or the undamaged roots.

4. Effect of A. avenae on Pre-emergence Damping-off of Bean Seedlings by Sclerotinia sclerotiorum

The use of mycophagous nematodes in the biological control of certain soil-borne plant pathogens has been investigated by several workers. Schindler and Stewart (1956) found that 3,200 Ditylenchus spp. per pot reduced wilt symptoms on carnation, due to Fusarium oxysporum f. dianthi. Rhoades and Linford (1959) observed that 125,000 A. avenae per pot controlled Pythium root-rot of corn. In a study of R. solani on beans, Barker (1964) showed that disease incitement was greatly reduced by high populations (100,000 nematodes per pot) of A. avenae. However, the above author pointed out that the population level necessary to give a high degree of control is not usually found under field conditions. Root-rot of beans and peas, caused by several fungi, was reduced considerably when populations of A. avenae were approximately 4,000-6,000/ml of fungus inoculum (Klink and Barker, 1968). The above experimental evidence indicates that A. avenae, in large numbers, will reduce or eliminate certain root diseases of plants. For this reason, the following experiment was carried out to determine whether A. avenae would reduce damping-off of bean seedlings caused by Sclerotinia sclerotiorum.

a. Methods and Materials

Forty clay pots (diameter 10 cm) were filled with a previously sieved (number 10 mesh, USS) sandy loam soil, and

autoclaved at 101.3kPa for 4 hr. When cool, depressions were made in the center of each pot to a depth of 2 cm and one bacteria-free Pencil Pod bean seedling was placed into each depression. Surface sterilization of bean seeds was carried out as in previous experiments.

S. sclerotiorum inoculum was grown on a modified Czapek solution in four 250 ml Erlenmeyer flask culture vessels. Each flask contained 25 ml of nutrient medium. The composition of the medium was as follows: $NaNO_3$, 2.0 g/l; K_2HPO_4, 1.0 g/l; KCl, 500 mg/l; $MgSO_4$, 500 mg/l; $CaSO_4$, 500 mg/l; $FeSO_4$, 10 mg/l; glucose, 20 g/l and distilled water to 1,000 ml. The flasks were inoculated with 1.0 ml homogenates of PDA cultured S. sclerotiorum, and after an incubation period of 6 days, the medium in each flask was completely occupied with dense mycelial growth. The cultures were then transferred to a sterile Waring blendor, and minced at slow speed for 1 minute. A one ml aliquot of the fungal suspension was pipetted over each of 32 bean seedlings.

Large numbers of A. avenae were extracted through Baermann funnels from cultures of S. bataticola. The nematodes were surface sterilized in 0.02% $HgCl_2$ for 15 min, and rinsed three times in sterile water. Using suitable dilutions, nematode concentrations of 13,200/ml, 6,600/ml and 3,300/ml were prepared. A one ml aliquot of each concentration was pipetted, immediately after addition of S. sclerotiorum, to each of the appropriate pots.

After inoculation was completed, soil was placed over each seedling, tamped down firmly and sterile water added. In order to prevent rapid moisture loss from the soil surface, each pot was covered with transparent polyethylene. The plastic was removed after 4 days, when seedling emergence was rapidly taking place. The pots were watered periodically as required.

Each treatment, replicated 8 times, was transferred to a plant growth chamber and randomized. Artificial illumination was supplied to the plants at 13,000 lx for 14 hr a day. The temperature of the air and soil during illumination was 21.5°C and 19.5°C respectively, whereas during the dark period, the temperature dropped to 18.5°C (air) and 16.5°C (soil). The length of the experiment was 31 days.

At this time, percentage seedling survival, plant height and fresh weight of tops and roots was determined.

b. Results

The results of this experiment are summarized in Table 20.

Twelve days after the experiment was initiated, unemerged seedlings in the S. sclerotiorum treatment were removed from the soil and examined for the presence of this fungus. Small black sclerotia and white mycelial tufts were found inside the damped-off seedlings. Small sections of diseased seedling tissue were surface sterilized in 0.01% $HgCl_2$ for 5 min,

rinsed three times in sterile water and plated on PDA. Characteristic mycelial growth and sclerotia formation of S. sclerotiorum occurred after several days. It was evident, therefore, that bean seedling damping-off was caused by S. sclerotiorum.

From the data summarized in Table 20 (which was not analyzed statistically due to the obvious treatment effects) it can be seen that pre-emergence seedling damage, caused by S. sclerotiorum, was eliminated at all inoculum levels of A. avenae. No effect on plant growth by the nematode was observed.

The above experiment was repeated, and, with the exception of a higher temperature of incubation and a slightly smaller nematode inoculum level, all treatments and materials used were the same as above. At a 3°C temperature increase (compared to the previous experiment) seedling pre-emergence damping-off by S. sclerotiorum did not occur. It was therefore not possible to determine whether A. avenae played a role in reducing the pathogenicity of this fungus, at the higher temperatures used. It was felt that because of the increased temperatures of soil and air, the bean plants were able to outgrow the mycelia of this fungus.

Table 20. Effect of A. avenae on emergence and growth of Pencil Pod beans colonized by S. sclerotiorum

Treatment	Percent Seedling Emergence	Height (cm)[a]	Fresh Wt. Tops (g)[a]	Fresh Wt. Roots (g)[a]
Control	100	20.0	2.5	2.2
S. sclerotiorum	12.5	21.2[b]	1.9[b]	2.4[b]
S. sclerotiorum + 3,300 A. avenae	100	20.5	2.6	2.7
S. sclerotiorum + 6,600 A. avenae	100	21.2	2.5	2.8
S. sclerotiorum + 13,200 A. avenae	100	21.2	2.6	2.6

[a]Average of eight replicates.

[b]Value represents one surviving replicate.

DISCUSSION AND CONCLUSIONS FOR PART II

Aphelenchus avenae exhibited selective tendencies for certain of the fungi tested, but the degree of attractiveness of each fungus depended on the time between nematode introduction and determination of the nematode response. Fungi which were preferred by A. avenae during the first 18 hr were apparently not as attractive to the nematode at 24 hr. An unidentified Fusarium sp. isolated from soil, Verticillium dahliae, and Gliocladium roseum, in that order, were the most attractive fungi at 18 hr. However, during the time between observations at 18 hr and 24 hr, fungal preference by A. avenae changed markedly, as indicated by the sharp increase in number of nematodes entering colonies of Verticillium albo-atrum, Fusarium culmorum, Verticillium cinnabarinum and Trichoderma viride (greatest to least, in order of attractiveness). The extreme difference between the average number of nematodes observed within the fungal colonies, which were evenly separated on each plate, showed that final choice was not random, but was governed by differences between the fungi tested. Although Pillai and Taylor (1967) concluded that factors governing fungus preference by mycophagous nematodes were unknown, work by Katznelson and Henderson (1964), Foreman (1968) and Klink et al., (1970), indicates that different chemical attractants, diffusible in agar, and produced in varying amounts by different fungi, served to attract A. avenae. Chemical

attractants were, therefore, probably responsible for the above observed distribution pattern. Prior to the 18 hr observation, then, the establishment by fungi of chemical gradients in the Water Agar (WA), was not extensive enough to attract A. avenae in large numbers. The rapid increase in numbers of A. avenae in colonies of certain fungi between 18 hr and 24 hr, however, reflects the response of this nematode to discrete regions of chemically attractive stimuli. On the other hand, the possibility of attractive volatile chemicals such as ethylene (Ilag and Curtis, 1968) cannot be overlooked.

The range of attractiveness demonstrated by the fungi tested could be interpreted on the basis of: (1) different diffusion rates of attractants in WA, (2) production of several attractive substances by certain fungi, and (3) variable outgrowth of mycelia from the PDA discs. Although no work has been done on diffusion rates of attractants in WA, Klink et al., (1970) found that several compounds chromatographed from culture filtrates of Gliocladium roseum could actively attract Neotylenchus linfordi. Liberation of more than one attracting substance by certain fungi may aid sensory perception of gradients by mycophagous nematodes thus resulting in aggregation of more nematodes in one colony than another. Mycelial growth from the inoculum discs into the WA did not occur due to the nutritionless nature of the medium. At 24 hr, none of the fungal colonies were visibly greater in diameter than the original 7 mm size, although aerial mycelial growth had developed in certain colonies. Therefore, variation observed in

nematode response to different fungi probably indicates basic physiological differences between fungal genera.

Attraction of a small number of A. avenae to control PDA discs is in agreement with the results of Klink et al., (1970), who recovered Neotylenchus linfordi in several instances from PDA discs, even when fungi were present on the same plate. This suggests that one or more substances in PDA are diffusible and slightly attractive to A. avenae.

None of the fungi tested were repellent to A. avenae. Nevertheless, at 24 hr, colonies of Sclerotinia sclerotiorum, Sclerotium bataticola and Helminthosporium sativum contained fewer nematodes than did the sterile PDA discs. The unattractiveness of these fungi could have been due to: (1) little or no release of attractive substances, (2) the production of repellents, or (3) the fact that the sensory mechanisms of A. avenae are sensitive only to a certain concentration range in a chemical gradient. Although Sclerotinia sclerotiorum and Sclerotium bataticola have not been included by other workers in mycophagous nematode host preference studies, Katznelson and Henderson (1964) found that, after an incubation period of 6 days, Helminthosporium sativum was distinctly attractive to Aphelenchoides parietinus. However, in view of the results of Pillai and Taylor (1967) and Klink et al., (1970), who demonstrated that nematodes respond to fungus colonies within 18 hr, the choice of 6 days by Katznelson and Henderson to

represent fungus attractiveness to A. parietinus may be questioned. Moreover, since these workers placed only one fungus colony on a plate opposite the nematode inoculum, it is highly probable that through food seeking and random movement, the nematodes could contact the fungus within 6 days. Even though Sclerotium bataticola was not attractive, this fungus was used routinely to build and maintain large populations of A. avenae for experimental work. Townshend (1964) found that Sclerotium rolfsii repelled A. avenae, and Agaricus hortensis was not attractive to this nematode. Katznelson and Henderson (1964) reported that colonies of Aspergillus niger, Myrothecium sp., Penicillium sp., and a Phoma sp. did not allow accumulation of many Aphelenchoides parietinus. Katznelson and Henderson's results, however, were obtained after 6 days of incubation whereas Townshend determined fungus host attractiveness at 18 hr. Katznelson and Henderson (1964) further observed that Aphelenchoides parietinus was not attracted to, nor did it reproduce on, Aspergillus niger while Mankau (1969a,b) noted that Aspergillus niger produced a toxic substance and did not support the growth of A. avenae. In this study, however, A. niger was a moderately attractive fungus to A. avenae. The difference in response to A. niger may be explained by different isolates of Aspergillus niger which vary in capacity to attract or repel A. avenae.

With the exception of Verticillium cinnabarinum, most of the fungi selected by A. avenae on WA were highly preferred

under soil conditions, although the order of preference changed considerably. Despite the fact that A. avenae and many of the fungi tested by other workers are soil-borne, no prior information could be found on the selective preferences of this nematode for fungi using soil as a medium. Verticillium cinnabarinum, Helminthosporium sativum and Sclerotium bataticola were not highly preferred by A. avenae. It would appear that H. sativum and S. bataticola do not produce attractant substances under agar or soil conditions. On WA V. cinnabarinum was highly attractive to A. avenae. However, the average number of A. avenae in and around colonies of this fungus on soil equalled the control PDA discs. This suggests that either the production of attractant substances was repressed by some substance(s) in the autoclaved soil, or the soil did not allow gradient establishment to occur.

None of the fungi tested, except a Pullularia sp., failed to support reproduction of A. avenae. However, considerable variation in final nematode numbers between fungi occurred after an incubation period of 16 days. Fusarium culmorum was clearly the most efficiently used host with progeny numbers reaching 22,470. Other fungi, namely Sclerotium bataticola, Fusarium sp., Trichoderma viride and Verticillium sp., were less efficient than F. culmorum, supporting 4,650, 2,315, 2,130 and 1,833 A. avenae, respectively. Nematode reproduction on Sclerotinia sclerotiorum and Verticillium dahliae was limited, with progeny totalling 910 and 725, respectively, while on a

Pullularia sp., recovery of A. avenae was less than the number of nematodes inoculated.

Although numerous studies have been made on the growth and reproduction of mycophagous nematodes on fungi, there is little information available on the reproduction of A. avenae on the fungi used in this study and no work has been done using soil as a medium for fungus growth. In their work with Ditylenchus destructor, Faulkner and Darling (1961) observed that reproduction at 9 weeks on PDA cultured fungi was as follows: Trichoderma viride, over 10,000; Verticillium albo-atrum from tomato, 100 to 1,000; V. albo-atrum from potato, over 10,000; Fusarium sp., over 10,000; a second Fusarium sp., 100 to 1,000; Aspergillus niger, no reproduction. Exact figures were not provided by these workers, nor was the method used for enumeration mentioned. Mankau and Mankau (1962) noted that Verticillium albo-atrum, and a Sclerotium sp. supported large populations of A. avenae while Trichoderma viride allowed only meager reproduction of the nematode. Numerical data was not presented by these authors, hence it is impossible to interpret the words "large" and "meager". Using a visual estimation technique for counting nematode numbers, Townshend (1964) found that after 2 weeks on PDA, multiplication of A. avenae was as follows: Fusarium sp. and Trichoderma viride, between 10,000 and 50,000; Gliocladium roseum, Verticillium albo-atrum and V. dahliae,

between 1,000 and 5,000. Although Pillai and Taylor (1967) studied the reproduction of A. avenae on various fungi, none of the fungi used by these workers were included in this study. Evans and Fisher (1970b) found that after 28 days on PDA, numbers of A. avenae reached 90,040 on Verticillium albo-atrum. Realistic comparison, however, of any of the numerical data on reproduction of A. avenae provided by the above workers, with data found in this study, is not possible due to widespread differences in: (1) length of incubation time, (2) temperature during incubation, (3) type of media, (4) nematode inoculum quantity, (5) size of culture dish (affects the amount of media available to the fungi), and (6) method of nematode enumeration.

Interpretation of the range in fecundity of A. avenae on different soil-cultured fungi is complicated by the interaction of several factors involving nematodes, fungi and the soil medium. The major factors which were operative in determining whether soil-grown fungi could support high or low numbers of A. avenae were: (1) the amount of mycelium produced by different fungi, and (2) the suitability of fungal protoplasm for nematode growth and reproduction. Those fungi which were capable of rapidly converting soil substrates into mycelial growth, and, because of their inherent genetic nature were suitable for conversion into nematode protoplasm, promoted the development of larger populations of A. avenae than fungi with lesser potential. Fusarium culmorum and Sclerotium bataticola not

only supported the highest reproduction of A. avenae, but, at the time of nematode inoculation, were observed to have produced abundant mycelial growth over the entire surface of the 60 mm soil plates. On the other hand, fewest numbers of A. avenae were recovered from colonies of Verticillium dahliae and Pullularia sp., which had grown only slightly within the 7 day incubation period prior to nematode inoculation. Observed differences in reproduction of A. avenae on different fungi may also have been due to: (3) inhibiting substances, formed naturally by different fungi or stimulated by nematode feeding, which retarded feeding, food digestion and reproduction of A. avenae, (4) hypersensitive reactions of certain fungi, similar to plants (Jenkins and Taylor, p 237, 1967), which, upon nematode feeding, act to delay or inhibit nematode reproduction, (5) complete destruction of all fungal mycelia (Riffle, 1967; Cooke and Pramer, 1968), thus preventing further nematode growth and development, (6) stimulation of fungal growth by nematode secretions administered through feeding, and (7) nematodes becoming quiescent in some cultures.

Three organisms, including the fungus Pullularia sp. and two unidentified actinomycetes, were nonhosts for A. avenae as indicated by recovery of fewer nematodes than originally inoculated. Due to its inability to divide rapidly in soil, and unicellular growth habit (Alexander, 1961), the Pullularia sp. did not provide A. avenae with a food supply sufficient

for reproduction. That A. avenae did not multiply on either of the actinomycete colonies supports the finding of Katznelson and Henderson (1964) who noted that Aphelenchoides parietinus did not reproduce on 56 isolates of soil actinomycetes. However, organisms which mycophagous nematodes cannot use as food, such as actinomycetes and bacteria, can nevertheless attract them strongly (Katznelson and Henderson, 1964).

Reproduction of A. avenae on different fungal hosts was influenced by time. The most rapid rates of nematode reproduction were recorded on Fusarium culmorum and Sclerotium bataticola. On most fungi, the number of A. avenae recovered was low at 4 days, and maximal at 8 days. Population growth declined quickly after 8 days. The nematodes required 12 days to reach total population growth on Fusarium sp. and Fusarium culmorum, before declining in numbers at 16 days. The above results suggest that population distribution over a time period is most likely due to factors governing population increase and decrease. Population increase is regulated by several factors including number of eggs laid per female, percentage of eggs hatched, and percentage of juveniles reaching adulthood (Pillai and Taylor, 1967). Since the number of A. avenae determined during the period of population increase differed with each fungus, it may be assumed that the quality and quantity of host subtrate determine the limit to which the above factors, suggested by Pillai and Taylor, can act to regulate population increase. In view of the statement of Evans and Fisher (1970b),

who suggested that egg production by A. avenae requires substantial amounts of nitrogen, and the requirement of this element for juvenile maturation, it would seem reasonable that termination of A. avenae reproduction and onset of population decline is due primarily to insufficient nitrogen available to support this nematode. Other nutritional factors, however, may also be involved. With the exception of Fusarium culmorum and Verticillium albo-atrum, population decrease of A. avenae proceeded rapidly on all fungi. The reasons for this may include: (1) unavailability of fresh food sources, (2) space limitations, (3) entry of nematodes into an anabiotic state, or (4) production of inhibiting substances by nematodes at high population densities. The latter is the most unlikely reason, however, because although the greatest population of A. avenae occurred on Fusarium culmorum, it had declined only slightly after 12 days, whereas on other fungi, and at much smaller densities, rapid population decline was observed as early as 8 days. The reproduction of A. avenae on Verticillium albo-atrum, unlike on the other fungi, was still increasing after the sixteenth day. This probably was due to the slow, sustained growth of the fungus in spite of continued nematode feeding.

The validity of amending sterilized soil with 0.5% glucose might be questioned because of the possibility of providing A. avenae with an extra nutritive factor sufficient

to affect reproduction. However, this is highly improbable since the average number of A. avenae extracted from control plates was always lower than the original inoculum.

The results of a preliminary study demonstrated that glucose has a stimulating effect on the growth of soil fungi, which indirectly affects the reproduction of A. avenae. Compared with unamended control soil, numbers of A. avenae were four times greater on Fusarium sp. and Trichoderma viride cultured on 1% glucose amended soil, while at 4% glucose levels, numbers of this nematode were approximately six times greater on Fusarium sp. and 17 times greater on T. viride.

A comparison of results on host attractiveness and host suitability indicate that a fungus preferred by A. avenae is not always the most suitable host for population increase. The maximum mean population level of A. avenae on Fusarium culmorum was 22,470 and this fungus was highly preferred both on agar and soil media. However, although Sclerotium bataticola supported the second highest number (4,650) of nematodes, this fungus, under the same conditions as F. culmorum, was the most unattractive of all hosts tested. These results confirm the conclusions of Pillai and Taylor (1967), notwithstanding the fact that the fungi tested by these workers were different from those used in this study.

Nodule formation on excised roots of beans was inhibited by A. avenae, indicating that this nematode can detrimentally

affect the interaction of Rhizobium phaseoli and its legume host. The degree of nodule reduction appeared to be dependent on the inoculum numbers. Little effect was shown by levels of 100 and 1,000 A. avenae, whereas 25.9% fewer nodules were formed on roots inoculated with 10,000 of these nematodes. The diminished number of nodules could have been due to: (1) the production, by high density A. avenae inoculum levels, of substances toxic to either R. phaseoli or bean roots, (2) impairment or restriction of bacterial infection thread development in root hairs, by feeding or probing of A. avenae, (3) stimulation of lateral rootlet formation, known to inhibit root hair infection (Nutman, 1965), (4) disturbance of normal host synthesis and secretion of products stimulatory to R. phaseoli, and (5) abnormally severe extrusion of protoplasm (Nutman, 1965) from apically weakened elongating root hairs, caused by a combination of host (Fahraeus and Ljunggren, 1968) and nematode injected polygalacturonase (Barker, 1966).

The observed difference in root fresh weight between A. avenae treatments and the uninoculated controls probably indicates nematode enhancement of excised root growth. Increased root growth may have been due to substances injected by the nematodes during feeding or released by the nematodes in response to root excretions. As a result, the activity of lateral root meristem areas may have been stimulated.

The average number (16) of nodules formed on roots inoculated with R. phaseoli was less than that reported by Cartwright (1967), who noted an average of 40 per excised root

system. The smaller number of nodules produced by excised roots in this experiment may reflect differences in the kind of bean cultivar used, texture of the root medium, or strain and time of addition, of R. phaseoli. In Cartwright's experiments, excised roots were obtained from a Black Wax cultivar of beans, grown vertically in a very coarse sand medium and inoculated twice with R. phaseoli. In this work, roots were removed from a Pencil Pod bean cultivar, grown in a horizontal position using a fine grain silica sand and inoculated only once with R. phaseoli.

Severe root hair and rootlet damage produced by A. avenae on agar-cultured excised bean roots suggests that the previously observed nodule reduction in the presence of this nematode is due mainly to destruction of root hairs and the nodulating sites immediately adjacent to centers of root hair infection. The results of this study not only conform with Chin and Estey's (1966) observations on root hair feeding by A. avenae, but, in view of the fact that solute absorption occurs most rapidly in the root-hair zone (Scott, 1965) and can be seriously affected in mutilated roots (Burstrom, 1965), the results probably indicate the mechanism responsible for the stunting and wilting noted by Chin and Estey on oat and cabbage plants. Detectable plant responses to A. avenae may, however, be influenced by several factors including the type of plant and the population level of the nematode. Chin and Estey (1966) noted that A. avenae did not feed on root hairs of 9 of 15 seedling plants tested and Barker (1964) was unable to detect

reduction in growth of beans in the presence of this nematode. Sutherland (1967) failed to detect any reduction in conifer seedling growth using 70 A. avenae, while Barker (1963) noted great reduction in growth of Kentucky Blue grass at an inoculum level of 2,000 nematodes per pot. Chin and Estey (1966) and Terry (1966) observed growth reduction and wilting of plants inoculated with 40,000 to 80,000 nematodes. However, Rhoades and Linford (1959) working with corn, Mankau and Mankau (1963) using orange seedlings, and Barker (1964) studying beans, did not observe any debilitating plant effects using as many as 100,000 to 125,000 nematodes per pot, although the pot sizes may have been quite different from those of the experiments referred to above. The validity of extrapolation from the results of experiments with excised roots to whole plants might be questioned on the basis of the inability of excised roots to perform normal root functions. However, studies by Raggio et al., (1957, 1959) indicate that isolated legume roots are capable of growth, root hair formation, nodule formation and nitrogen fixation, functions which are characteristic of normal legume roots. The presence of few nematode eggs and the colorless appearance of adult A. avenae at 18 days suggest that, in spite of frequently observed food uptake from root hair and epidermal rootlet cells, this nematode was unable to obtain the necessary nutrients for egg production and juvenile maturation. The lack of color demonstrated by adult A. avenae resembles the starved appearance of A. avenae sieved from soil

in which corn had been growing (Rhoades and Linford, 1959). Although no reports have been published showing reproduction of A. avenae on healthy higher plants, these results, together with the discovery of root hair feeding by Chin and Estey (1966), and the reports of A. avenae being found within roots (Christie and Arndt, 1936; Steiner, 1936; Barker, 1964; Terry, 1966) indicate that under certain conditions, this nematode can feed on and enter the roots of higher plants. This disagrees with the conclusions of Sutherland (1967) and Sutherland and Fortin (1968) who claimed that A. avenae not only cannot feed on roots of higher plants but is unable to enter healthy tissues of higher plants. Sutherland and Fortin (1968) admitted, however, that A. avenae may enter roots providing that a pathogenic fungus has preceded the nematode. This might explain the presence of A. avenae in roots of plants grown under greenhouse conditions. Entry of A. avenae into plant roots could also have been aided by liberation of cellulase and polygalacturonase by large numbers of A. avenae (Barker, 1966) which weakened the normally resistant epidermal cell layer.

The increased activity exhibited by A. avenae in the presence of R. phaseoli indicates nematode stimulation either directly by R. phaseoli or indirectly through substances released by interacting roots and cells of the bacterium. The nature of the stimulus is not known but is most likely chemical and may involve the profuse polysaccharide secretions known to occur in different strains of Rhizobium (Fahraeus and Ljunggren,

1968; Alexander, 1971), or compounds excreted by the roots in response to liberation of bacterial substances. The stimulus is probably not highly specific for any one nematode function since several responses including movement, feeding, and egg laying were observed. The stimulated activity of A. avenae during the first 7 days in the presence of R. phaseoli and agar cultured excised bean roots, and the movement of adult nematodes away from the severely damaged roots at 11 days, suggest that the stimulus is effective when excised roots are relatively undamaged. The production of eggs by A. avenae was notably influenced by the presence of R. phaseoli. Although several hatched juveniles were observed feeding on plant cells, maturation did not occur, probably due to the damaged root hairs, root caps and rootlet surfaces. These results strengthen the hypothesis of Katznelson and Henderson (1964) who suggested that the rhizosphere microflora not only may have a profound effect on soil nematodes, but this effect may be completely unrelated to the food value of the microflora.

Nematodes were observed to be most numerous and active in cloudy films or zones surrounding the submerged rootlets. These cloudy areas, which probably contained R. phaseoli cells, were frequented by constantly moving, feeding, and egg laying adults of A. avenae. It is possible that the stimulus which promoted heightened nematode activity was formed and retained in the slightly opaque zone adjacent to the roots. The transformation at 7 days, of this continuous amorphous zone into a

particulate or granular, non-cloudy appearance, could have been due to ion complexes which formed as a result of interaction between nematode, root, and bacterial excretions, and the mineral substances of Cartwright's inorganic medium suspended in the agar. Since nodule formation in beans occurs via bacterial root hair infection, the deficiency in nodule numbers in these excised root experiments may have been due to the poor root hair development of the roots growing in the agar medium (Raggio et al., 1957).

Damping-off of beans incited by Sclerotinia sclerotiorum, was controlled completely by all inoculum levels of A. avenae tested. The already established fact that A. avenae can feed and multiply on soil cultured S. sclerotiorum, the use of homogenized mycelial suspensions as inoculum, and the lack of damping-off in nematode treated plants, indicate that this nematode is capable of either total destruction of all mycelial inoculum or reduction of the inoculum level below that required for root infection. Although numerous studies have shown that A. avenae can reduce root diseases caused by fungi (Rhoades and Linford, 1959; Barker, 1964; Klink and Barker, 1968) the effectiveness of A. avenae appears to depend mainly on the type of plant and the inoculum level of the nematode. The results support the conclusion of Rhoades and Linford (1959), that under controlled environment conditions, A. avenae is a beneficial nematode.

PART III

STUDIES OF MELOIDOGYNE HAPLA

1. The Effect of Bean Cultivar on Penetration and Development of M. hapla

Despite numerous studies of the physiology of root-knot nematode-host relations, very little work has been done on the effects of different host cultivars on the morphological development of this nematode. This is especially true for the early stages of root parasitism. Barrons (1939) found that juvenile root-knot nematodes penetrated the roots of resistant and susceptible beans in equal numbers. Later, however, Barrons observed heavy galling on the susceptible cultivar and light galling on the resistant cultivar. Crittenden (1958) studied the reactions of soybean cultivars to Meloidogyne incognita acrita and noted that the number, size and morphology of giant cells differed between resistant and susceptible cultivars. Blazey et al., (1964) showed that several bean cultivars which were resistant to M. incognita were highly susceptible to M. hapla and two other root-knot nematodes. Reynolds et al., (1970) demonstrated that juvenile root-knot nematodes migrated out of the root systems of resistant alfalfa cultivars, whereas in susceptible cultivars, juveniles became sedentary and developed normally.

The objective of this part of the present research was to determine the effect of different bean cultivars on the

growth and development of Meloidogyne hapla Chitwood during the early stages of nematode morphogenesis.

a. Methods and Materials

Soaked seeds of 11 bean cultivars were surface sterilized in 0.1% $HgCl_2$, rinsed three times in sterile water and plated on modified CRMA. Bean cultivars used were Romano, Unrivalled Wax, Brittle Wax, Pencil Pod, Cherokee Wax, Puregold, Contender, Bountiful, Tendergreen, Highlander and Kentucky Wonder Wax. Contaminant free seedlings were transferred to culture tubes (20 mm x 150 mm) of sandy loam soil previously autoclaved for 55 min at 101.3 kPa. One seedling was placed into a depression in the soil of each tube.

Egg masses of M. hapla were removed from roots of infected tomato plants, and surface sterilized in 0.12% NaOCl for 15 min. The surface sterilized egg masses were then transferred to Petri dishes containing sterile water, and incubated for 5 days. Hatched juveniles were collected and 1.0 ml aliquots containing approximately 750 nematodes were pipetted to each seedling. The seedlings were covered with sterile soil and given 5 ml of sterile water. The tubes were then wrapped with aluminum foil and placed in a growth cabinet. Artificial illumination at 13,000 lx was supplied to the plants for 14 hr per day. The air temperature of the cabinet was 22°C throughout the experiment.

At intervals of 7, 14 and 21 days, two inoculated plants of each cultivar were removed from the incubator, and the roots washed, fixed, and stained for nematode size determination. The roots were fixed in FAA for at least 24 hr before staining in 0.05% cotton blue lactophenol at 80°C for 1 min. Destaining was carried out using clear lactophenol. Young roots stained light blue, whereas the older parts of the root system stained less deeply. Root tips appeared a deep blue.

To determine the effect of each bean cultivar on M. hapla development, the roots were carefully dissected, using teasing needles, and nematode size and shape were compared to standards originally proposed by Christie (1946). A total of 100 nematodes were examined. The standard shapes (Fig. 11) are described as follows: Group A, includes juveniles from the stage where growth has begun to the stage where they still possess a more or less conical tail; Group B, includes juveniles from the stage with a more or less hemispherical posterior end, terminated by a spike, to the stage where they are about to complete the final molt; Group C, includes females from the stage of molt completion to the stage where they are almost fully grown; Group D, includes fully grown females (or nearly so) that have not laid eggs; and Group E, includes egg-laying females.

b. Results

The results of this experiment are presented in Tables 21, 22 and Figures 12, 13.

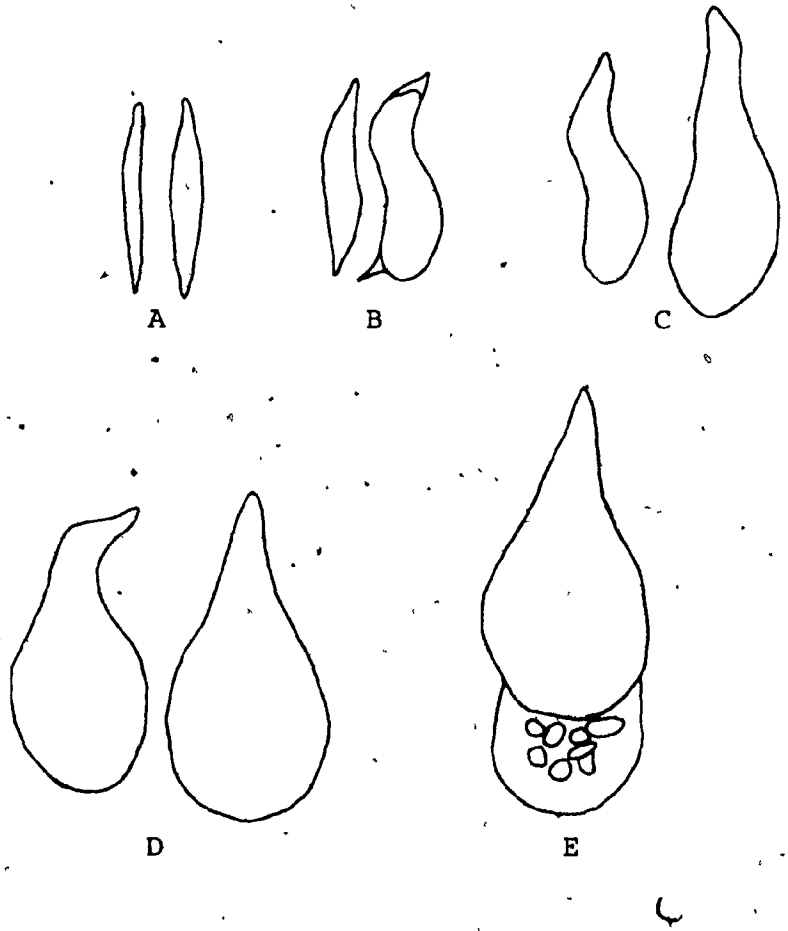


Figure 11. Root-knot nematode developmental stage classification (after Christie, 1946).

Table 21. Effect of bean cultivar on development of M. hapla 14 days after inoculation

Cultivar	Developmental Stage[2] of M. hapla					
	A[3]	B[3]	C[3]	D[3]	E[3]	Total
Bountiful	4.5[a][1]	95.5[a][1]	0	0	0	100
Brittle Wax	5.5[a]	94.5[a]	0	0	0	100
Cherokee Wax	7.5[a]	20.5[a]	0	0	0	28
Contender	5.0[a]	58.5[a]	0	0	0	63.5
Highlander	5.5[a]	94.5[a]	0	0	0	100
Kentucky Wonder Wax	6.5[a]	93.5[a]	0	0	0	100
Pencil Pod	11.5[a]	88.5[a]	0	0	0	100
Puregold	15.0[a]	73.5[a]	0	0	0	88.5
Romano	8.0[a]	92.0[a]	0	0	0	100
Tendergreen	19.0[a]	69.5[a]	0	0	0	88.5
Unrivalled Wax	8.5[a]	91.0[a]	0.5	0	0	100

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]See Figure 11 for illustrations of the developmental stages of M. hapla.

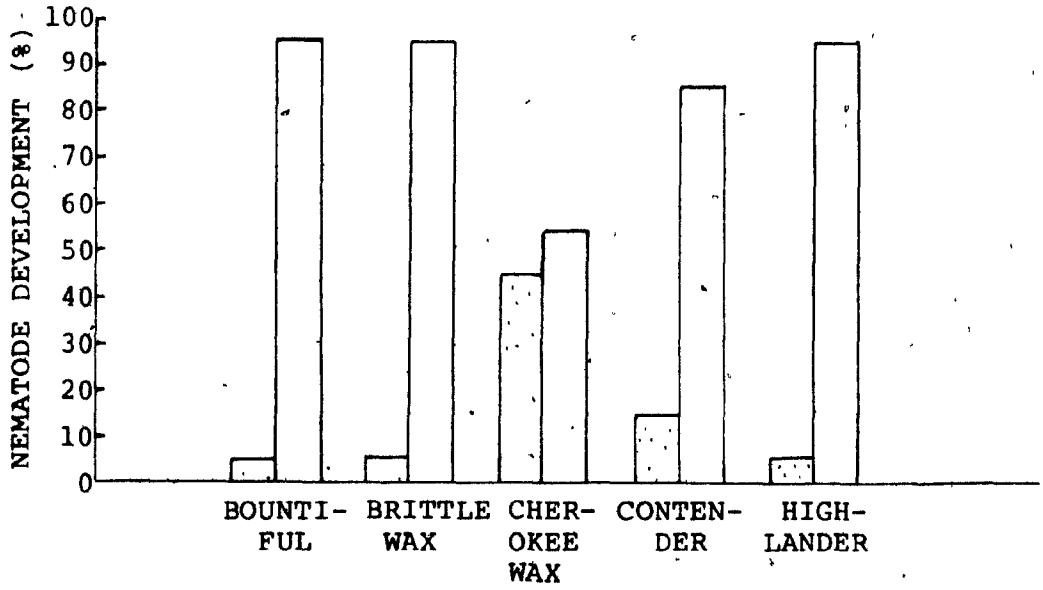
[3]Each number represents the average of two replicates.

After an incubation period of 7 days, visible evidence in the form of tiny root swellings, of nematode penetration and development, could not be observed. Root dissection revealed small numbers of recently penetrated second stage juveniles which had undergone little development. Since nematode

development after one week remained within Group A, only the data for the second and third week of incubation are reported.

Nematode development between 7 and 14 days after inoculation was rapid (Table 21). The majority of nematodes had reached the Group B stage, characterized by spike-terminated, hemispherical ends. Numbers of M. hapla at the A and B stages of morphogenesis varied between cultivars; however, the differences were not significant (Appendix Tables XVIII, XIX). Cherokee Wax (Figure 12), allowed less root penetration than any of the cultivars tested. With the exception of only one of several hundred nematodes examined, development did not reach the Group C stage.

Nematodes continued to develop and increase in size until the third week. Considerable, but not statistically significant (Appendix Tables XX, XXI, XXII), differences between cultivars were observed at this time. Four bean cultivars, including Unrivalled Wax, Puregold, Contender and Bountiful, appeared to be the most resistant to nematode growth and development (Table 22 and Fig. 13). In each of the above cultivars the greatest percentage of nematodes examined had reached only the Group B stage. These cultivars, however, were not completely resistant. In each case a small percentage of nematodes developed to the Group D stage. The cultivars allowing greatest percentage of Group D stage development were Kentucky Wonder Wax, Brittle Wax, Highlander, Romano and Tendergreen. No cultivar supported nematode development to the egg laying stage (Group E) in this three week period.



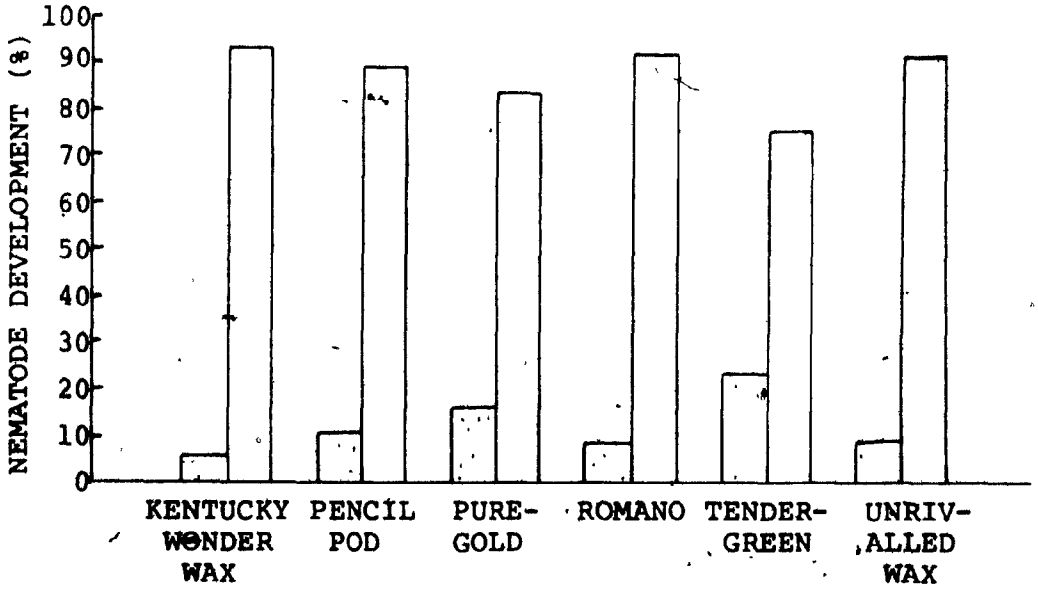


Figure 12. Developmental stage of M. hapla on different bean cultivars 14 days (average of two replicates) after inoculation.

Group A Group B

Table 22. Effect of bean cultivar on development of M. hapla 21 days after inoculation

Cultivar	Developmental Stage[2] of M. hapla					
	A[3]	B[3]	C[3]	D[3]	E[3]	Total
Bountiful	0.5	47.5[a][1]	36.0[a][1]	16.0[a][1]	0	100
Brittle Wax	0.5	15.5[a]	46.0[a]	38.0[a]	0	100
Cherokee Wax	0	29.0[a]	46.0[a]	25.0[a]	0	100
Contender	0	54.5[a]	19.5[a]	26.0[a]	0	100
Highlander	0	23.5[a]	37.0[a]	39.5[a]	0	100
Kentucky Wonder Wax	0.5	28.0[a]	30.5[a]	41.0[a]	0	100
Pencil Pod	0	33.0[a]	35.5[a]	31.5[a]	0	100
Puregold	0	52.5[a]	38.5[a]	9.0[a]	0	100
Romano	0	32.5[a]	28.0[a]	39.5[a]	0	100
Tendergreen	0	30.0[a]	32.5[a]	37.5[a]	0	100
Unrivalled Wax	0	42.0[a]	33.0[a]	25.0[a]	0	100

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]See Figure 11 for illustrations of the developmental stages of M. hapla.

[3]Each number represents the average of two replicates.

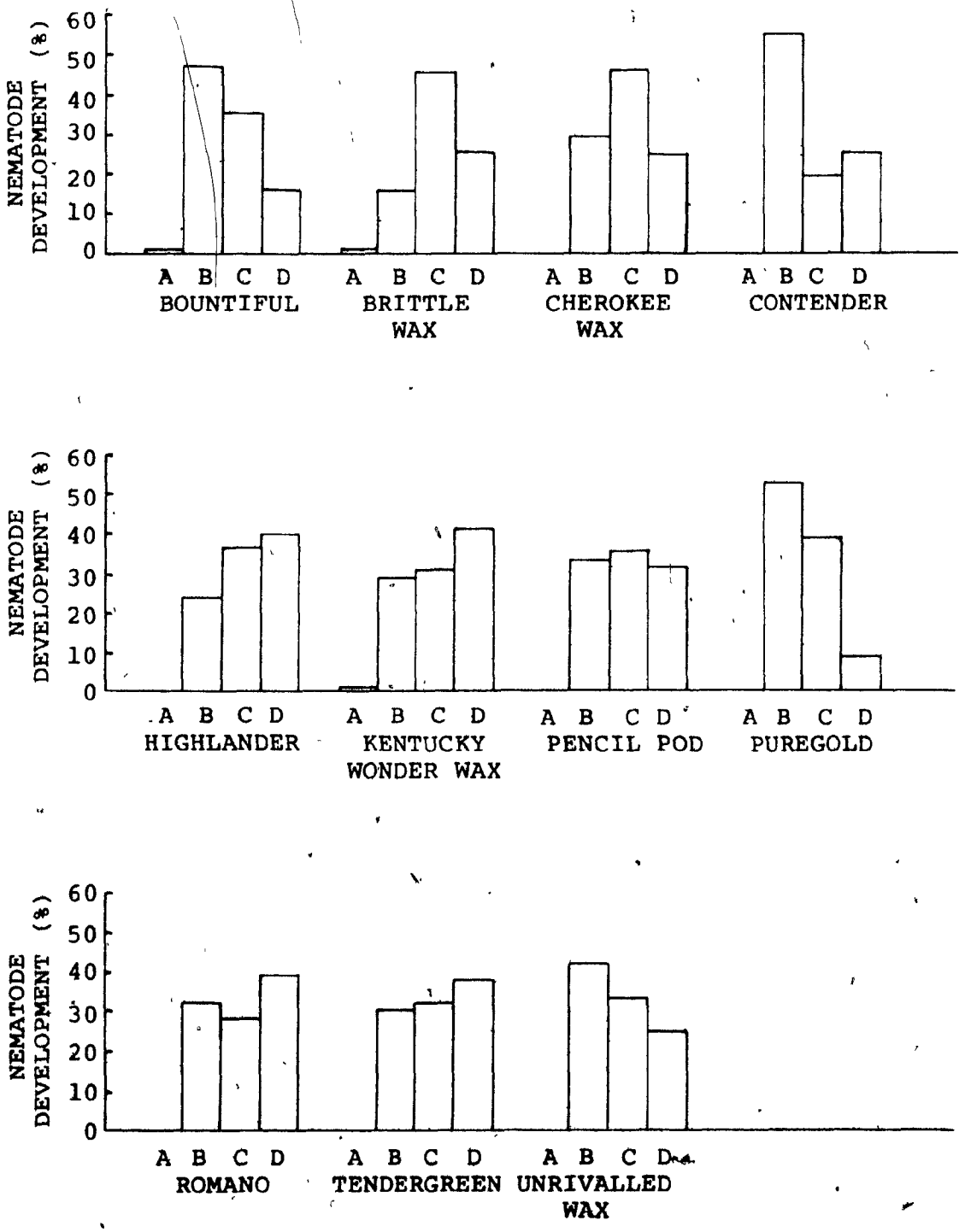


Figure 13. Developmental stages of M. hapla on different bean cultivars 21 days (average of two replicates) after inoculation.

2. The Effect of Soil Yeasts on Penetration and Development of M. hapla on Beans, Peas and Clover

It is well known that certain fungal and bacterial members of the soil microflora affect the parasitism of plant roots by root-knot nematodes. To date, however, studies have not been made on the effect of soil yeasts on root-knot nematode-plant interactions, despite documented evidence of the existence of yeasts in soil (di Menna, 1957; Alexander, 1961; Babeva and Saveleva, 1963; Babeva and Belyanin, 1966; Last and Price, 1969).

In this part of the present research, a study was undertaken to determine the effects of different soil yeasts on the penetration and development of M. hapla on beans, peas, and clover.

a. Methods and Materials

Soaked seeds of beans (Slendergreen), peas (Little Wonder) and red clover (Dollard) were surface sterilized in 0.1% $HgCl_2$, rinsed three times in sterile distilled water and plated on modified CRMA. Germinated seedlings, free of contaminating microorganisms, were transferred to cotton-plugged 250 ml Erlenmeyer flasks of greenhouse soil, sterilized 24 hr before seedling addition. One seedling was placed into a depression made in the soil of each flask by a sterile glass rod.

Around the root system of each plant was pipetted a two ml suspension containing approximately 2,000 juveniles of M.

hapla. The juveniles were obtained from surface sterilized egg masses (gently agitated for 10 min in 0.12% NaOCl (commercial bleach, Javex). After rinsing twice in sterile distilled water, the egg masses were transferred to sterile screwcap culture tubes and violently hand shaken for 3 min to dislodge the eggs from the gelatinous matrix. Dislodged eggs and pieces of egg mass material containing embedded eggs were transferred to sterile Petri plates containing 15 ml sterile distilled water, and left to hatch at room temperature.

Yeasts were cultured on a modified Wickerham (1951) medium. This consisted of glucose, 10 g/l; malt extract, 3.0 g/l; yeast extract, 3.0 g/l; peptone, 5.0 g/l and distilled water, 1,000 ml. Fifty ml of the above medium were placed in cotton-plugged 250 ml Erlenmeyer flasks and autoclaved at 101.3 kPa for 15 min. Upon cooling, the flasks were inoculated with Candida mycoderma, Lodder and Kreger-van Rij, Rhodotorula pallida, Lodder and Torulopsis magnoliae, Lodder and Kreger-van Rij, and incubated at 25°C, on a rotary shaker, for 3 days. A five ml suspension of the required yeast culture was pipetted to the root zone of appropriate plants.

Exposed seedling roots were then covered with sterile soil and given 10 ml sterile water. The soil portions of the flasks were wrapped with aluminum foil and placed in a temperature controlled cabinet. The plants were illuminated with artificial light at 13,000 lx for 14 hr a day. The temperature of the air during illumination was 23°C, and 18°C during the

dark period. The length of the experiment was 28 days. Sterile water was added periodically as required.

The treatments, each replicated six times, were as follows:

1. Beans + *M. hapla*
2. Beans + *M. hapla* + *T. magnoliae*
3. Beans + *M. hapla* + *R. pallida*
4. Beans + *M. hapla* + *C. mycoderma*
5. Peas + *M. hapla*
6. Peas + *M. hapla* + *T. magnoliae*
7. Peas + *M. hapla* + *R. pallida*
8. Peas + *M. hapla* + *C. mycoderma*
9. Clover + *M. hapla*
10. Clover + *M. hapla* + *T. magnoliae*
11. Clover + *M. hapla* + *R. pallida*
12. Clover + *M. hapla* + *C. mycoderma*

Due to the rapid growth of the bean and pea seedlings, the elongating seedling hypocotyls quickly contacted the cotton stoppers, and pushed them from the Erlenmeyer flasks. To prevent microbial contamination, a piece of sterile cotton was fitted evenly around the elongating hypocotyl. Because of the slower growth rate of the clover seedlings, contact with the cotton stopper did not occur until the termination of the experiment.

Upon completion of the experiment, plant roots were washed carefully, fixed in FAA for 24 hr and stained in 0.05%

cotton blue lactophenol at 80°C for 1 min. Destaining was omitted since portions of the root system containing nematodes did not stain deeply.

Roots were carefully dissected, using teasing needles, and nematode maturity was determined according to the method of Christie (1946). All nematodes in each root system were observed for size, shape and presence of eggs.

b. Results

The results of this experiment, designed to study the effects of different yeasts on the penetration and development of M. hapla on beans, peas and red clover, are summarized in Tables 23, 24, 25 and Figures 14, 15, 16, 17, 18 and 19.

The total number of M. hapla dissected from axenic plant roots varied with the type of plant (Table 23, 24, 25). Peas supported the largest number, 217.6; beans, 133.8; and red clover, only 12.5. Probably the most tenable reason for such a low number of nematodes in red clover roots is the slow growth rate of the plants.

In general, fewer M. hapla entered and developed in plant roots treated with yeast inoculum (Fig. 14). With the exception of red clover, which did not produce a vigorous, rapidly extending root system (the data in Table 25 were not analyzed statistically due to apparent lack of treatment effects and number of dead plants), the number of M. hapla in roots of

Table 23. Effect of soil yeasts on penetration and development of M. hapla on beans

Treatment	Development[2] of M. hapla						
	A	B	C	D	E	♂	Total
M. hapla	0.5[3]	39.7[a1]	28.5[a1]	56.5[a1]	6.5[a1]	2.2	133.8[a1]
M. hapla + T. magnoliae	0.2	22.5[ab]	20.8[a]	36.5[a]	6.2[a]	3.2	89.3[a]
M. hapla + R. pallida	0	14.5[b]	15.6[a]	17.3[a]	10.5[a]	3.0	61.0[a]
M. hapla + C. mycoderma	0.2	18.2[ab]	17.1[a]	26.5[a]	11.8[a]	3.5	77.3[a]

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]See Figure 11 for illustrations of the developmental stages of M. hapla.

[3]Average of six replicates.

beans and peas treated with either T. magnoliae, R. pallida or C. mycoderma was less than the controls. In beans (Table 23), numbers of M. hapla counted in roots treated with T. magnoliae, C. mycoderma and R. pallida were 33.2%, 42.2% and 54.5% lower, respectively, than in uninoculated control plants (treatment differences were not significant, Appendix Table XXIII). A similar pattern was observed in peas (Table 24), with lower numbers (not statistically significant, Appendix Table XXIV) of M. hapla entering and developing in roots treated with yeasts, than in control plants.

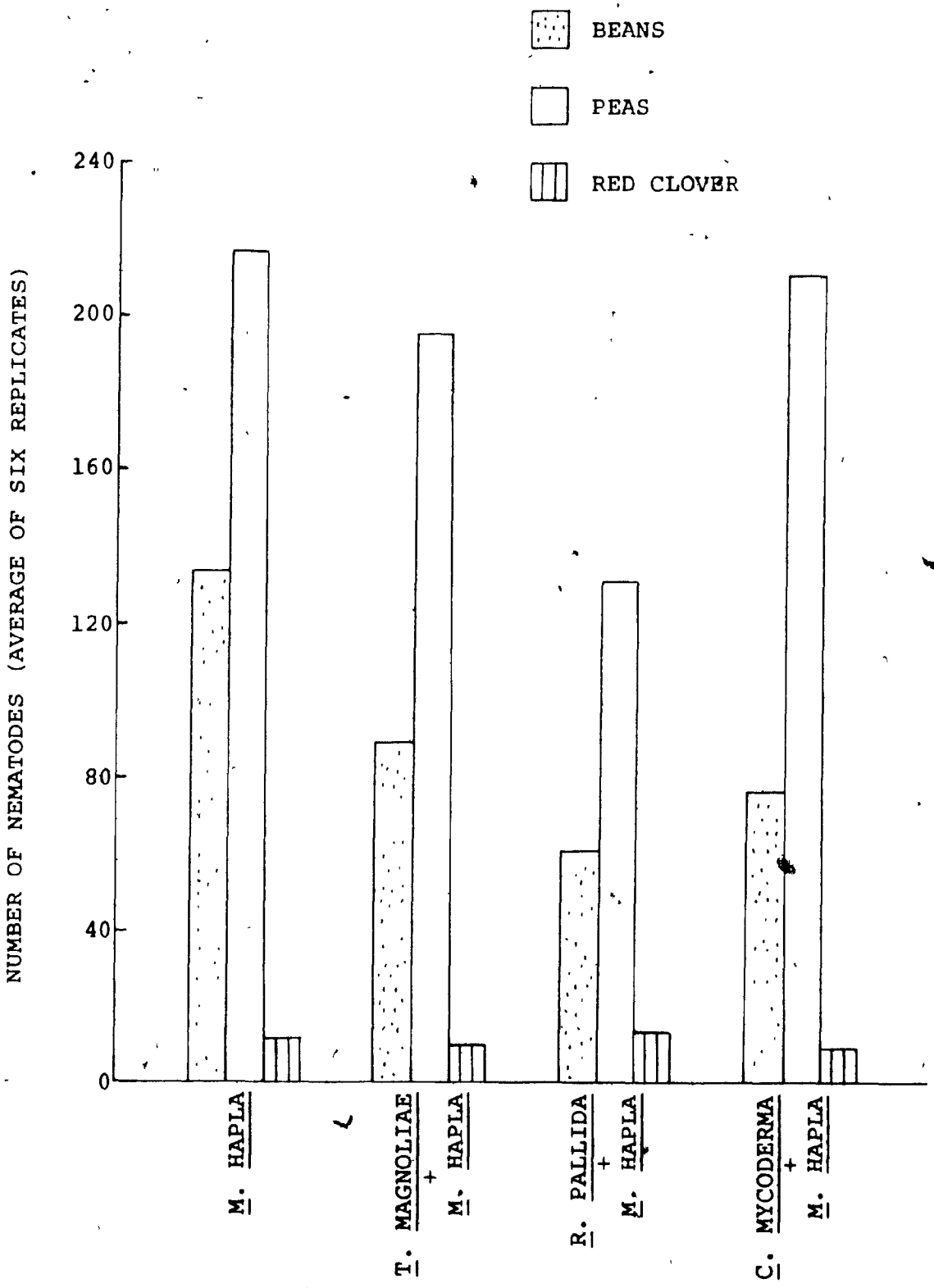


Figure 14. Effect of soil yeasts on the number of M. hapla developing in the roots of beans, peas and red clover.

Table 24. Effect of soil yeasts on penetration and development of M. hapla on peas

Treatment	Development[2] of M. hapla						
	A	B	C	D	E	♂	Total
M. hapla	2.8[3]	108.5[a1]	35.5[a1]	52.0[ab1]	19.0[a1]	0.2	217.6[a1]
M. hapla + T. magnoliae	3.6	96.0[ab]	50.4[a]	34.0[b]	12.4[a]	0.2	196.6[a]
M. hapla + R. pallida	1.5	45.7[b]	38.5[a]	33.0[b]	12.5[a]	0	131.2[a]
M. hapla + C. mycoderma	0.7	58.8[ab]	33.2[a]	86.8[a]	31.8[a]	0.3	211.5[a]

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]See Figure 11 for illustrations of the developmental stages of M. hapla.

[3]Average of six replicates.

Growth and development of M. hapla, as affected by different yeasts and plants, can be observed in Tables 23, 24, 25 and in Figures 15, 16, 17, 18, and 19.

Very few nematodes, which entered the roots of peas and beans, were still at the A stage of development after 28 days (Tables 23, 24 and Fig. 15). There was no observable effect of yeast treatment on M. hapla development in these plants, hence analysis of variance was not carried out. In red clover

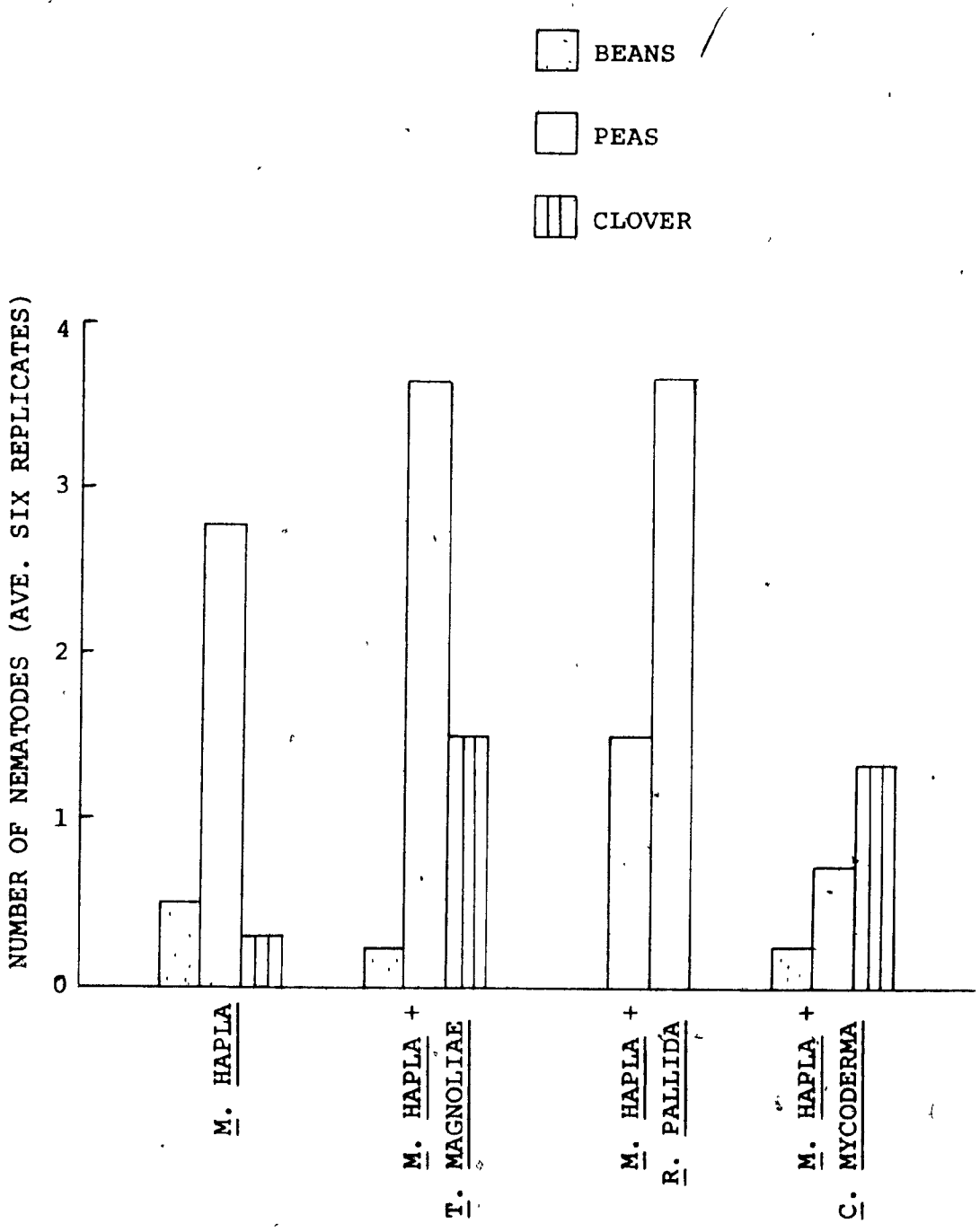


Figure 15. Number of M. hapla at the A stage of development after 28 days.

Table 25. Effect of soil yeasts on penetration and development of M. hapla on clover

Treatment	Development[1] of M. hapla						
	A	B	C	D	E	♂	Total
M. hapla	0.3[2]	7.8	1.2	2.5	0.5	0.2	12.5
M. hapla + T. magnoliae	1.5	8.0	0.5	0.3	0	0	10.3
M. hapla + R. pallida	3.7	10.0	0.3	0	0	0	14.0
M. hapla + C. mycoderma	1.3	8.3	0.7	0	0	0	10.3

[1]See Figure 11 for illustrations of the developmental stages of M. hapla.

[2]Average of six replicates.

(Table 25) the number of M. hapla in roots treated with different yeasts was higher than in the control treatment.

In contrast to the small numbers of M. hapla found at the A stage of development, the largest percentage, in general, of any level of maturation of M. hapla, was the B stage (Fig. 16). In red clover, the effect of yeast inoculum was similar to that of the A stage above with larger numbers of M. hapla in all yeast treatments (R. pallida, 28.0%; T. magnoliae, 3.0%; C. mycoderma, 6.0%), compared with the control.

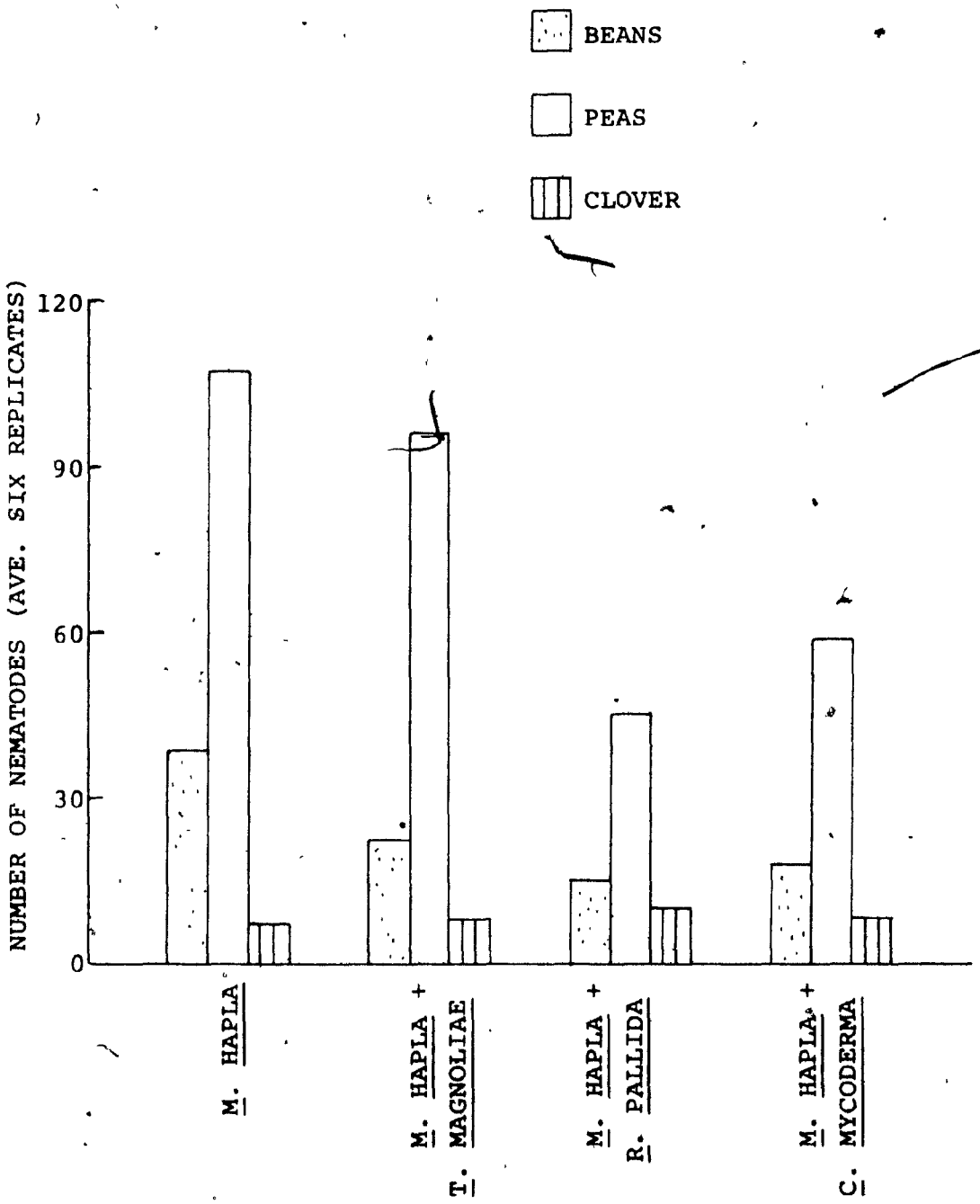


Figure 16. Number of M. hapla at the B stage of development after 28 days.

In beans and peas, however, the opposite result was observed. In beans, significantly fewer (Appendix Table XXV) M. hapla at the B stage were counted in roots treated with R. pallida, whereas lower, but not statistically significant, numbers were found in roots treated with T. magnoliae and C. mycoderma. Similarly, in peas, significantly lower numbers (Appendix Table XXVI) of B stage M. hapla were determined in R. pallida treated roots, while lower (not significant) populations were counted in roots treated with T. magnoliae and C. mycoderma.

Fig. 17 illustrates the number of M. hapla at the C stage of growth. Nematodes reaching this stage have completed the final molt and are almost fully grown. It was noted that yeast-treated red clover plants supported fewer M. hapla than the control. This complete reversal from the A and B stages, in which plants inoculated with yeasts supported greater numbers of M. hapla compared to the control was also noticed in the D and E stages (Fig. 18 and 19). The number of C stage M. hapla in all yeast inoculated beans and peas (Table 23, 24) was not significantly different (Appendix Table XXVII, XXVIII) than controls, indicating that these soil yeasts did not affect the development of nematodes at this stage.

The number of M. hapla at the D stage of growth is shown in Fig. 18. Although fewer D stage nematodes were found in bean roots treated with yeasts, compared with control plants

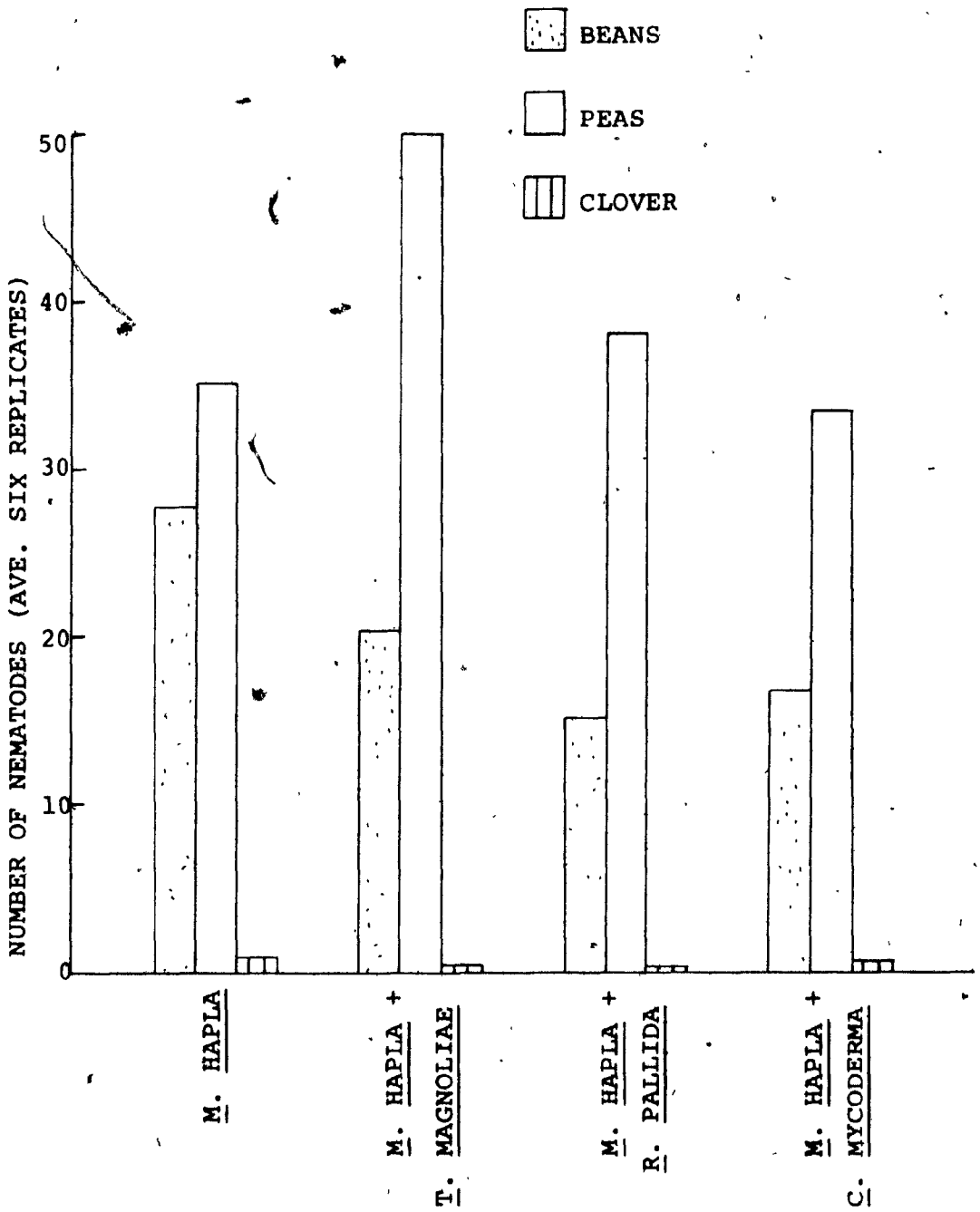


Figure 17. Number of M. hapla at the C stage of development after 28 days.

(Table 23), the differences were not significant (Appendix Table XXIX). In peas, there were significantly greater numbers (Appendix Table XXX) of D stage M. hapla in roots treated with C. mycoderma, than in controls, whereas fewer (not significant) nematodes were counted in roots inoculated with T. magnoliae and R. pallida.

Few nematodes matured on either peas, beans or red clover, to the egg-laying stage (Tables 23, 24, 25 and Figure 19). Bean plants treated with yeast inoculum contained slightly higher numbers (not statistically significant, Appendix Table XXXI) of E stage M. hapla than the controls. In peas, fewer M. hapla were counted in roots treated with T. magnoliae and R. pallida, whereas greater numbers were observed in C. mycoderma inoculated roots. Differences due to treatments were not, however, statistically significant (Appendix Table XXXII) than the controls. Numbers of E stage M. hapla in red clover were considerably lower in all yeast treatments. From the data presented in Fig. 17, 18, 19, it is clear that the necessary requirements for growth and development to the egg-laying stage, were not present in red clover plants treated with different yeasts.

Nodules were absent in all plants examined. The number of mature male nematodes observed was slightly greater in beans (Table 23) than in peas and red clover (Tables 24, 25), but did not seem to be influenced by any of the yeast treatments.

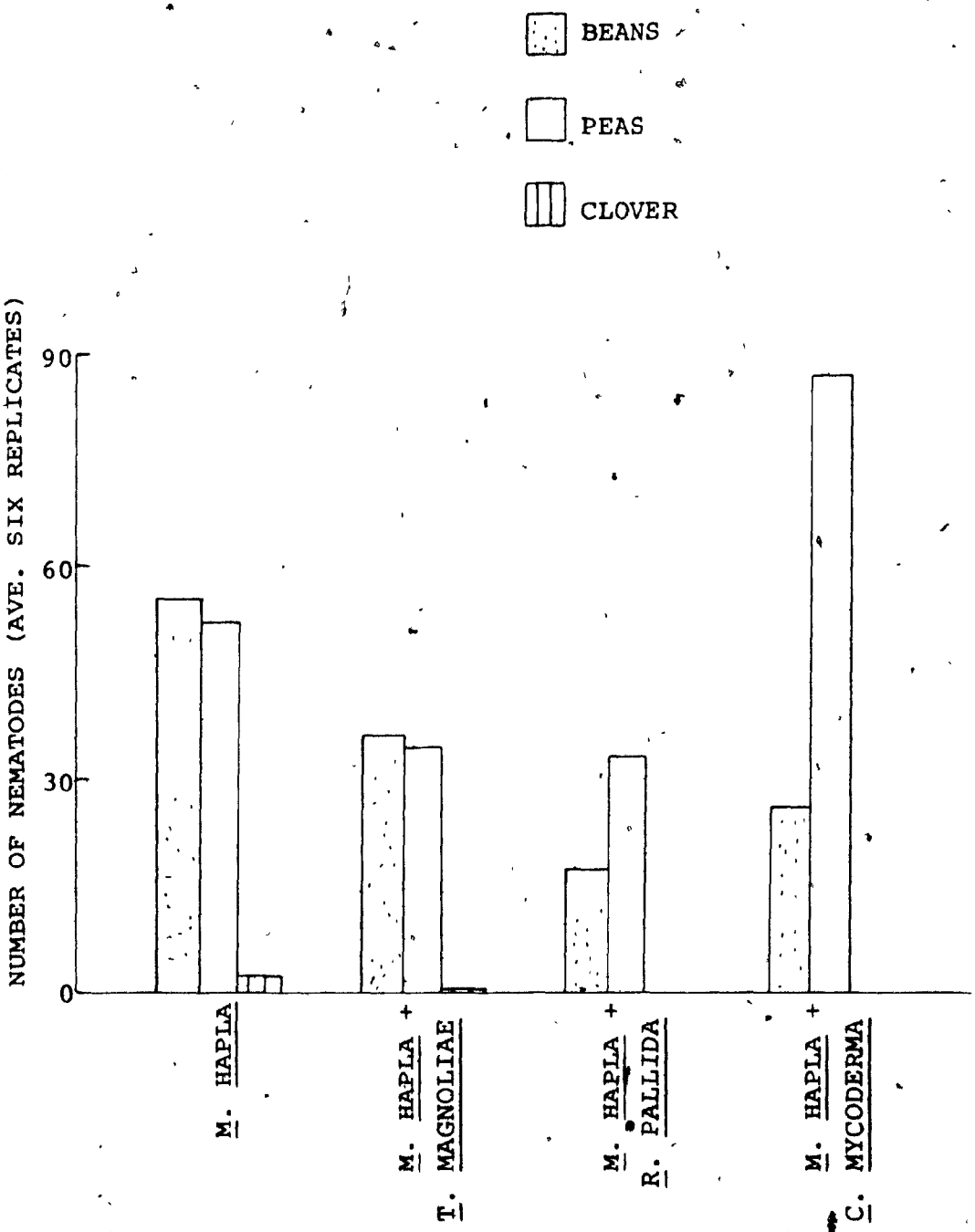


Figure 18. Number of M. hapla at the D stage of development after 28 days.

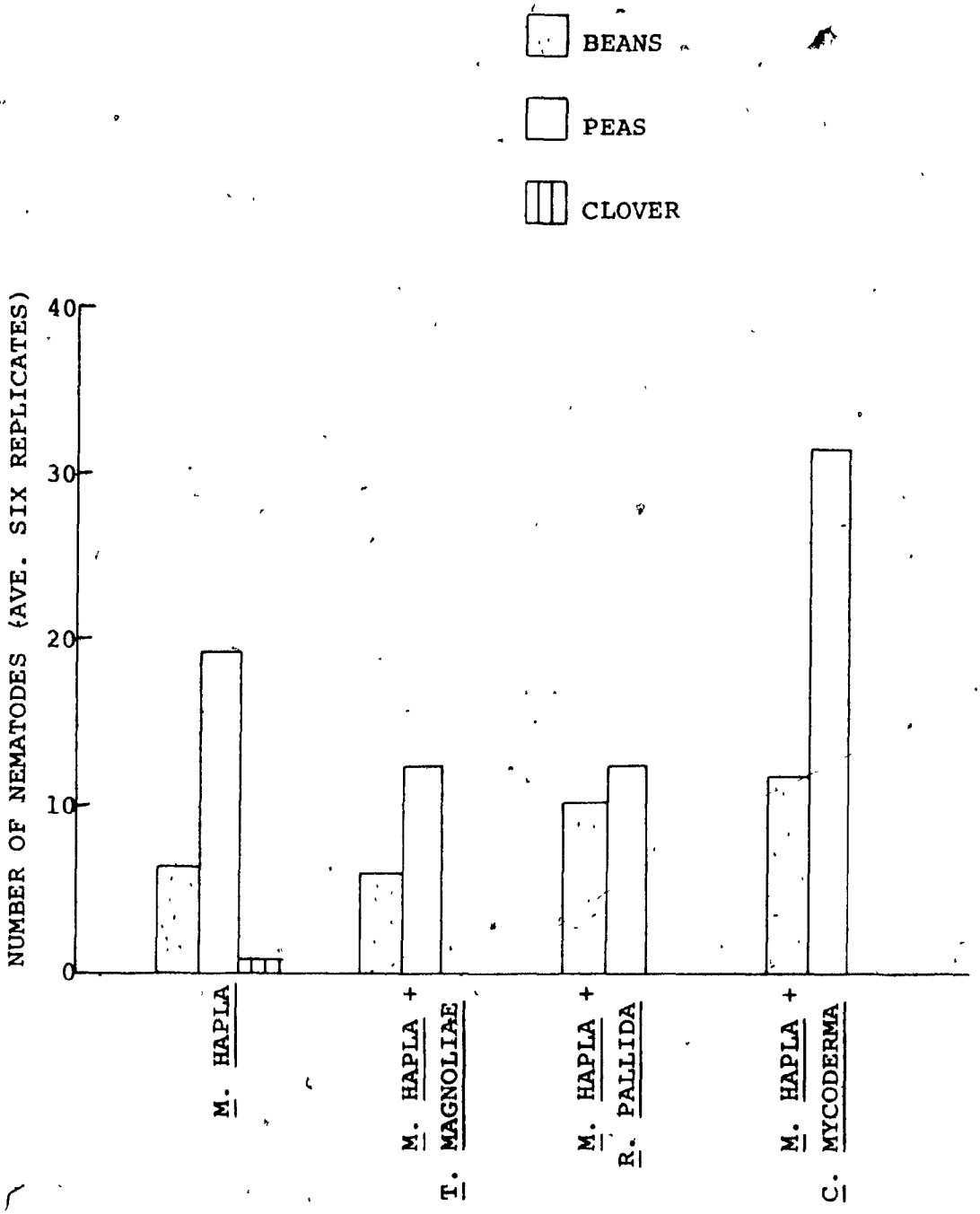


Figure 19. Number of M. hapla at the E stage of development after 28 days.

Since there were no apparent differences between treatment means, analysis of variance was not completed for this form of M. hapla development.

3. The Effect of Soil Fungi Upon Root Invasion and Egg Hatch of M. hapla

The majority of studies involving associations of nematodes, fungi and plants have been concerned with the effects of parasitic nematodes on plant diseases caused by soil-borne fungi. Such studies have shown that addition of nematodes to plants infested with disease inducing fungi caused: (1) earlier disease symptom appearance (Mountain, 1965), (2) greater yield reduction (Powell, 1971b), and (3) more rapid loss of plant disease resistance, than in fungus inoculated plants alone (Powell, 1963).

Complex interaction of nematodes and saprophytic fungi has been noted by Edmunds and Mai (1966,a) who showed that higher numbers of Pratylenchus penetrans were recovered from alfalfa roots, three days after treatment with varying spore concentrations of Trichoderma viride and Fusarium oxysporum, than were recovered from noninoculated roots. In another study Edmunds and Mai (1966,b) observed that the population of P. penetrans recovered from alfalfa roots after a period of eight weeks, was significantly larger in roots infected with a combination of P. penetrans and T. viride than in treatments with nematodes alone.

In the present research, experiments were designed to test the effect of fungus inoculated bean and pea roots on the

amount of galling by M. hapla. An experiment testing the influence of soil-borne fungi on egg hatch of M. hapla was also conducted.

a. Effect of Different Soil Fungi on Root Invasion of Beans by M. hapla

(i) Methods and Materials

Procedures for this experiment were as follows. Fresh field soil was sieved through a USS number 10 mesh sieve and placed into each of forty-eight 500 ml wide mouth Erlenmeyer flasks.. The flasks were stoppered with cotton, autoclaved for three hours and left in the laboratory to aerate for three days.

Fungus inocula of Trichoderma viride, Pers. ex Fr., Fusarium roseum, Link and an unidentified species of Penicillium, Link were grown in 25 ml of a modified Czapek solution in 250 ml Erlenmeyer flask culture vessels. The composition of the medium was the same as described in Part II, Section 4. After a period of six days, four cultures of each fungus were homogenized for 30 seconds in a small sterile metal blender, and 5 ml of this spore-mycelia suspension were pipetted to depressions made in the soil of certain units.

Juveniles of M. hapla were obtained from surface sterilized egg masses. Approximately 1,500 nematodes were pipetted in 1 ml volumes to the required treatments.

Axenic, freshly germinated Pencil Pod bean seedlings were then placed into soil depressions and covered with sterile soil. As the seedlings emerged from the flasks sterile cotton was fitted around the plant stems, thereby preventing contamination of the root system.

The experiment consisted of eight treatments, each replicated six times. The treatments were as follows:

1. Bean plants
2. Bean plants + *T. viride*
3. Bean plants + *F. roseum*
4. Bean plants + *Penicillium* sp.
5. Bean plants + *M. hapla*
6. Bean plants + *T. viride* + *M. hapla*
7. Bean plants + *F. roseum* + *M. hapla*
8. Bean plants + *Penicillium* sp. + *M. hapla*

All plants were given 20 ml of sterile distilled water and randomized in a growth cabinet. Prevention of root exposure to light was accomplished by enclosing the flasks with aluminum foil. The plants were illuminated at 13,000 lx for 14 hours. The soil temperature during the light period was 23°C and 17°C during the dark period. Sterile water was added periodically as required. Due to the rapid plant growth it was necessary to provide support by inserting sterile straight wire rods into the soil of each flask.

The plants were harvested after 28 days. Fresh weights of the above-ground plant portions and of roots were taken and the number of nematode galls were counted.

(ii) Results

The effect of different soil-borne fungi on the amount of root galling of beans by M. hapla is summarized in Table 26.

Galling of bean roots was 56% greater per gram root fresh weight and 34% greater per plant in T. viride treated roots (not significant statistically, Appendix Tables XXXIII and XXXIV). In plants treated with F. roseum the number of galls per gram of root, incited by M. hapla, was 56% lower (not significant) than uninoculated roots, whereas on a per plant basis, galling was 47% lower (statistically significant) than control roots. A slight decrease (nonsignificant) in galling was noted in bean roots treated with Penicillium sp., compared with control roots.

Root growth was retarded by all organisms, alone or combined. Growth of shoots, however, was increased by most treatments but especially those including either M. hapla alone or in combination with a fungus.

b. The Effect of Trichoderma viride on Root Invasion of Beans by M. hapla

(i) Methods and Materials

The methods used in soil preparation, fungus and nematode inoculum preparation, seed surface sterilization and treatment

Table 26. Effect of M. hapla and various fungi on weight and root galling of beans

Treatment	Fresh Weight (g)[2] Foliage	Roots	Root Galling[2] Number/Plant	No./g Root Fr.Wt.
Control	4.4	3.2	0[c1]	0[c1]
T. viride	4.7	2.5	0[c1]	0[c]
F. roseum	4.7	2.4	0[c]	0[c]
Penicillium sp.	4.0	2.0	0[c]	0[c]
M. hapla[3]	6.7	3.0	104.5[a]	34.7[ab]
T. viride + M. hapla	7.0	2.7	140.6[a]	54.1[a]
F. roseum + M. hapla	6.0	2.5	55.6[b]	22.2[b]
Penicillium sp. + M. hapla	6.7	2.6	89.1[ab]	34.2[ab]

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]Average of six replicates.

[3]1,500 nematodes/flask as inoculum.

replication were the same as described above.

Five ml suspensions of T. viride, consisting of spores and minced mycelia, were delivered to soil depressions in certain 500 ml Erlenmeyer flasks. Germinated Pencil Pod bean seeds were planted immediately after the T. viride treatment. Plant growth, and colonization of the soil by T. viride, was allowed to take place for a period of nine days. At this time 3,000 nematodes were added in 1 ml of sterile water around the plant roots.

The experiment consisted of four treatments, which were as follows:

1. Bean plants
2. Bean plants + T. viride
3. Bean plants + M. hapla
4. Bean plants + M. hapla + T. viride

All treatments were randomized in a greenhouse and watered when necessary with sterile distilled water. The average soil temperature during the day was 25°C and 20°C during the night.

The experiment was terminated after 42 days. Fresh weight data of foliage and roots was obtained and the number of galls on each root system determined.

In a second experiment, the conditions of which were essentially the same as above, slight changes were made in nematode and fungus inocula, number of replicates and duration of plant growth.

Ten ml aliquots of minced spore-mycelia suspensions of T. viride were dispensed to soil depressions as above. After nine days 4,000 juveniles of M. hapla were applied in 10 ml of water. Treatments were replicated six times and the plants were harvested after 28 days.

(ii) Results

In the first experiment the number of galls per gram of root on a fresh weight basis, and on a per plant basis, was significantly greater (Appendix Tables XXXV, XXXVI) in roots

infected with a combination of M. hapla and T. viride than in treatments with nematodes alone (Table 27). This relationship held true in the second experiment (Table 28), but not to the same extent, as the number of galls per gram of root on a fresh weight basis was greater, but not significantly (Appendix Table XXXVII), in roots treated with M. hapla and T. viride compared with nematode treated controls. On a per plant basis, fewer galls (statistically nonsignificant, Appendix Table XXXVIII) formed in roots treated with the combined nematode-fungus inoculum than in those receiving nematodes alone.

Table 27. The effect of M. hapla and T. viride on weight and root galling of beans

Treatment	Fresh Weight (g)[2]		Root Galling[2]	
	Foliage	Roots	Number/Plant	No./g Root Fr.Wt.
Control	10.8	5.7	0[c][1]	0[c][1]
T. viride	12.0	5.2	0[c]	0[c]
M. hapla[3]	8.8	5.0	287[b]	57.4[b]
T. viride + M. hapla	10.0	5.0	521[a]	104.2[a]

[1]Any two means in a column with the same superscript are not significantly different (P≤0.05).

[2]Average of six replicates.

[3]3000 nematodes/flask as inoculum.

Table 28. The effect of 4,000 M. hapla and T. viride on weight and root galling of beans

Treatment	Fresh Weight (g)[2] Foliage	Roots	Root Galling[2] Number/Plant	No./g Root Fr.Wt.
Control	9.3	5.0	0[b1]	0[b1]
T. viride	12.5	5.0	0[b]	0[b]
M. hapla	8.3	4.8	486[a]	101.2[a]
T. viride + M. hapla	8.4	4.0	452[a]	113.0[a]

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]Average of six replicates.

c. The Effect of Sclerotinia sclerotiorum on Root Invasion of Peas and Beans by M. hapla

(i) Methods and Materials

Greenhouse steamed soil was placed into 32 clay pots (diameter 10 cm) previously autoclaved for 30 minutes at 101.3kPa. When cool, the pots were taken to the laboratory and depressions were made in the center of each pot to a depth of 2 to 3 cm. One Pencil Pod bean seed, surface sterilized as described in previous experiments, was placed into each depression.

Four mycelial mats of Sclerotinia sclerotiorum, (Lib.) de Bary, grown in a liquid basal medium as in the above experiments, were homogenized in a sterile blender and 1 ml aliquots of the mycelial suspension were pipetted over the seed surface.

One thousand juveniles of M. hapla, collected from surface sterilized egg masses, were added in 1 ml of sterile water immediately after addition of the fungus.

The treatments, replicated seven times, consisted of the following:

1. Bean plants
2. Bean plants + S. sclerotiorum
3. Bean plants + M. hapla
4. Bean plants + S. sclerotiorum + M. hapla.

The pots were kept in a growth chamber maintained at 23°C during the light period and 17°C during the dark period, with a cycle of 14 hours light (13,000 lx) and 10 hours darkness. The study was terminated 42 days after seedling inoculation.

As in the previous experiments, fresh weight data was obtained for root foliage growth and the number of galls were counted.

In a second experiment, the objective was to repeat the above experiment but with peas, to determine whether S. sclerotiorum would influence the amount of galling by M. hapla on Little Wonder peas. The amount of homogenized mycelial inoculum added was 10 ml and the number of root-knot nematodes delivered to each pot was 2,000. All other variables were the same as in the previous experiment.

(ii) Results

The number of galls per plant was 84% more extensive (statistically significant, Appendix Table XXXIX) in bean roots inoculated with M. hapla and S. sclerotiorum than in the nematode treatment alone (Table 29). Although galling was 34% greater, on a per gram root fresh weight basis, in roots receiving the combined inoculum treatment compared with those inoculated with nematodes alone, the difference was not significant (Appendix Table XL).

Table 29. The effect of M. hapla and S. sclerotiorum on weight and galling of beans

Treatment	Fresh Weight (g)[2] Foliage	Roots	Root Galling[2] Number/Plant	No./g Root Fr.Wt.
Control	17.4	2.7	0[c][1]	0[b][1]
S. sclerotiorum	16.5	2.3	0[c]	0[b]
M. hapla[3]	14.9	3.3	204.5[b]	62.1[a]
S. sclerotiorum + M. hapla	19.6	4.5	373.6[a]	83.0[a]

[1]Any two means in a column with the same superscript are not significantly different (P≤0.05).

[2]Average of seven replicates.

[3]1,000 nematodes/pot as inoculum.

In the experiment with peas, the phenomenon of increased galling in the presence of S. sclerotiorum was again manifested (Table 30). The number of galls per gram of root on a fresh weight basis was 118% more extensive (statistically significant, Appendix Table XLI) in roots inoculated with M. hapla and S. sclerotiorum than in nematode treated roots. On a per plant basis, the number of galls was 64% greater in roots treated with the combined inocula compared with roots receiving M. hapla alone. The difference, however, was not significant statistically (Appendix Table XLII). Shoot growth of peas was severely reduced by M. hapla and M. hapla plus S. sclerotiorum, whereas root growth was not influenced by any treatment. Gall formation on pea roots was far more extensive than it was on beans.

Table 30. The effect of M. hapla and S. sclerotiorum on weight and galling of peas

Treatment	Fresh Weight (g)[2] Foliage	Roots	Root Galling[2] Number/Plant	No./g Root Fr.Wt.
Control	5.6	1.0	0[b1]	0[c1]
S. sclerotiorum	5.0	1.0	0[b]	0[c]
M. hapla[3]	1.7	1.2	530[a]	442[b]
S. sclerotiorum + M. hapla	1.0	0.9	868[a]	964[a]

[1]Any two means in a column with the same superscript are not significantly different (P≤0.05).

[2]Average of eight replicates.

[3]2,000 nematodes/pot as inoculum.

d. The Effect of Soil Fungi on the Egg Hatch of M. hapla

Increased root galling by M. hapla in the presence of two soil fungi was clearly shown in the previous experiments. To test the effect of one of these and other soil-fungi on nematode egg hatch the following experiment was conducted.

(i) Methods and Materials

Two percent Difco agar was poured into fifty sterile 60 x 15 mm Petri dishes and allowed to solidify. Using sterile technique a 10 mm circular disc of autoclaved Whatman No. 1 filter paper was suspended into either a blended mycelial-spore suspension of T. viride, F. roseum, Penicillium sp., Sclerotium bataticola Taub., or sterile distilled water and transferred to the center of one of the above Petri dishes.

Ten surface sterilized egg masses of M. hapla were then placed onto each of the above fungus treated or untreated discs. The treatments, replicated ten times, are listed below.

1. Egg masses on untreated sterile discs
2. Egg masses + T. viride
3. Egg masses + F. roseum
4. Egg masses + Penicillium sp.
5. Egg masses + S. bataticola

The plates were incubated in a dark chamber kept at 25°C. After four days, the number of juveniles hatched from each group of ten egg masses was determined using a stereomicroscope.

(ii) Results

The results of this experiment are given in Table 31. The number of juveniles emerging from egg masses of M. hapla varied with the treatment. Compared with uninoculated controls, egg hatch was greater in treatments with Penicillium sp., T. viride, and F. roseum by 25%, 38% and 68% respectively. The only fungus to cause a significant increase in egg hatch was F. roseum (Appendix Table XLIII). Egg mass treatment with S. bataticola resulted in a 25% decrease (not statistically significant) in egg hatch, compared with the controls.

Table 31. Influence of treatment with S. bataticola, Penicillium sp., T. viride and F. roseum on egg hatch of M. hapla

Treatment	Juveniles Hatched/[2] Ten Egg Masses
Control[3]	742[bc][1]
S. bataticola	554[c]
Penicillium sp.	938[abc]
T. viride	1024[ab]
F. roseum	1248[a]

[1]Any two means in a column with the same superscript are not significantly different (P≤0.05).

[2]Average of ten replicates.

[3]Surface sterilized egg masses plus sterile discs.

4. Effect of M. hapla on Nodulation of Beans by R. phaseoli

The effects of root-knot nematodes on legume-rhizobia relationships have recently received attention from plant pathologists. A study completed by Shands and Crittenden (1957) showed that Rhizobium may increase the pathogenicity of M. incognita acrita on soybeans. In assessing the role of root-knot nematodes in legume nodulation, Masefield (1958) reasoned that nematode galls impaired nodulation in two ways: (1) chemically, by utilizing plant nutrients at the expense of nodule bacteria, and (2) physically, by occupying sites normally available for nodules. Crittenden (1962) observed that nodule formation, on a nodulating strain of soybean, was reduced by treatment with M. incognita acrita. Complete inhibition of nodule formation on hairy vetch by M. hapla was noted by Malek and Jenkins (1964). Nigh (1966) found that alfalfa nodulation was reduced 50% by M. javanica when treatments were applied at the time of planting and by 30% when rhizobia inoculation was delayed 32 days. Nigh discovered that the amount of galling was reduced, and not increased, in the presence of Rhizobium. Taha and Raski (1969) showed that M. javanica did not significantly reduce the number of nodules per gram of white clover root. They noted that nodule reduction was due to reduced root growth and not the direct effect of nematode treatment. Panayi (1970) found that nodule formation in beans was reduced by treatment with M. hapla.

For the present study, experiments were designed to determine the effect of M. hapla on nodule formation of beans by R. phaseoli. In order to avoid nodulation of uninoculated control plants as experienced in experiments reported in section one, and by Panayi (1970), bean roots were grown under more carefully controlled sterile environments.

a. Whole Plant Experiments

(i) Methods and Materials

Each of forty-eight 250 ml Erlenmeyer flasks were given 260 g of a dry soil-sand mixture, prepared by mixing three parts of an air dried field soil with one part No. 24 silica sand. The sand was added to facilitate better aeration of the soil. The flasks were then fitted with cotton plugs, and autoclaved for 2½ hours. The final pH of the autoclaved and cooled soil was 6.6.

Egg masses of M. hapla were pipetted to the soil surface at the rate of 10, 50 or 100 per unit depending on the treatment, after they were surface sterilized in 0.12% NaOCl (commercial bleach, Javex) for 5 minutes and rinsed once in sterile water. Using a sterile glass rod the egg masses were mixed into the top 5 cm of soil. Egg hatch in sterile water was determined to be approximately 2,000 juveniles in 10 egg masses, 10,000 in 50 egg masses, and 20,000 in 100 egg masses.

Inoculum of Rhizobium phaseoli, Dangeard was prepared from streaked slants on congo red mannitol agar (CRMA). Five ml aliquots were pipetted onto the surface of appropriate treatments.

Germinated Slendergreen bean seeds, free from contamination, were then placed into a depression made in the soil of each flask. The soil was replaced over each seed.

Sterile distilled water was added immediately after bean seeds and inoculum treatments. The amount of water was calculated to bring the soil to 60% of its moisture holding capacity.

The experiment, consisting of eight treatments, each replicated six times, lasted for 31 days. The treatments were as follows:

1. Bean plants
2. Bean plants + 10 egg masses
3. Bean plants + 50 egg masses
4. Bean plants + 100 egg masses
5. Bean plants + R. phaseoli
6. Bean plants + 10 egg masses + R. phaseoli
7. Bean plants + 50 egg masses + R. phaseoli
8. Bean plants + 100 egg masses + R. phaseoli

Maintenance of a sterile root environment was ensured by inserting sterile 125 ml long-neck Erlenmeyer flasks (the neck portion being wrapped with a thin piece of cotton) into the necks of the 250 ml flasks when each seedling contacted the cotton stopper. After the rapidly elongating hypocotyl had entered the smaller inverted Erlenmeyer flask, sterile cotton was packed evenly around the plant stem.

Prevention of root exposure to light was accomplished by wrapping the basal portion of each unit with aluminum foil. All units were randomized in a greenhouse where plants were given 13,000 to 16,000 lx of supplemental light. The temperatures of the greenhouse were approximately 22 to 25°C during the day and 17 to 20°C during the night.

At the end of the experiment root fresh weight data was obtained and nodulation on each root system was determined.

In a second experiment, the effect of adding R. phaseoli five and ten days after M. hapla treatment, on the nodulation and root growth of beans was studied.

Details of soil preparation, seed surface sterilization, treatment replication, sterile root environment maintenance, and conditions of plant growth were the same as described above.

A ten ml suspension containing approximately 5,000 juveniles of M. hapla was pipetted evenly over the surface of previously moistened soil in appropriately designated vessels. Germinated bean seeds were immediately placed on the soil surface, covered with sterile silica sand, and moistened with sterile water. Five ml aliquots of R. phaseoli were delivered to the correct treatments after five and ten days. The experiment, which lasted 29 days, consisted of the following treatments, each replicated six times:

1. Bean plants
2. Bean plants + M. hapla

3. Bean plants + R. phaseoli after 5 days
4. Bean plants + R. phaseoli after 10 days
5. Bean plants + M. hapla + R. phaseoli after 5 days
6. Bean plants + M. hapla + R. phaseoli after 10 days

In a third experiment conditions were essentially the same as in experiment two, except that a smaller nematode inoculum was used and rhizobia inoculation was made ten days after the addition of nematodes. Approximately 3,000 juveniles in ten ml aliquots were used as nematode inoculum. This experiment, consisting of four treatments, each replicated seven times, lasted for 24 days. The treatments were as follows:

1. Bean plants
2. Bean plants + M. hapla
3. Bean plants + R. phaseoli
4. Bean plants + M. hapla + R. phaseoli

(ii) Results

The results of the experiments outlined above are summarized in Tables 32, 33 and 34.

From the data presented in Table 32, it can be seen that simultaneous addition of R. phaseoli and M. hapla, compared with R. phaseoli alone, resulted in increased nodulation of bean roots. Plants treated with R. phaseoli and 10 egg masses had an average increase in number of nodules of 71% per plant and 89% per gram root fresh weight (neither of these differences were significant, Appendix Tables XLIV, XLV). With an inoculum

Table 32. The effect of 10, 50, and 100 egg masses of M. hapla on the nodulation of beans

Treatment	Av. Root Fresh Wt.(g)[2]	Av. No. Nodules[2]	Av. No. Nodules /g Root Fr.Wt.[2]
Control	3.16	0^{b}[1]	0^{c}[1]
10 egg masses	1.80	0^{b}	0^{c}
50 egg masses	1.94	0^{b}	0^{c}
100 egg masses	2.24	0^{b}	0^{c}
R. phaseoli	2.23	40.8^{a}	17.4^{b}
R. phaseoli + 10 egg masses	2.05	70.0^{a}	33.0^{ab}
R. phaseoli + 50 egg masses	2.15	78.5^{a}	36.3^{a}
R. phaseoli + 100 egg masses	1.91	56.0^{a}	28.9^{ab}

[1]Any two means in a column with the same superscript are not significantly different (P≤0.05).

[2]Average number of six replicates.

of 50 egg masses per plant, the average increase in nodules per plant was 92% (not significant), whereas the number of nodules per gram root fresh weight increased 108% (statistically significant). The highest nematode inoculum (100 egg masses) also increased the average number of nodules per plant (37%) and per gram root fresh weight (66%), although the increase was not significant.

Table 33. The effect of M. hapla and delayed rhizobial inoculation on nodulation of beans

Treatment	Av. Root Fresh Wt.(g)[2]	Av. No. Nodules[2]	Av. No. Nodules /g Root Fr.Wt.[2]
Control	2.48	0[b][1]	0[b][1]
M. hapla	2.24	0[b]	0[b]
R. phaseoli 5 days	2.95	60.8[a]	20.6[a]
R. phaseoli 10 days	2.75	43.6[a]	15.8[a]
M. hapla + R. phaseoli 5 days	2.87	65.8[a]	22.9[a]
M. hapla + R. phaseoli 10 days	2.60	56.0[a]	21.6[a]

[1]Any two means in a column with the same superscript are not significantly different (P≤0.05).

[2]Average number of six replicates.

Increased nodulation was also observed in bean plants treated with rhizobia five and ten days after infestation with nematodes (Table 33). Plants receiving rhizobia five days after M. hapla treatment showed an average increase in number of nodules of 8% per plant and 11% per gram root fresh weight. The treatment differences were not statistically significant, however (Appendix Tables XLVI, XLVII). Similarly, but to a greater degree, in plants receiving R. phaseoli ten days after M. hapla treatment, the average increase in nodules per plant

Table 34. The effect of inoculation of beans with R. phaseoli after 10 days exposure to M. hapla

Treatment	Av. Root Fresh Wt. (g)[2]	Av. No. Nodules[2]	Av. No. Nodules[2] /g Root Fr. Wt.
Control	2.33	0^{b}[1]	0^{b}[1]
M. hapla	2.37	0^{b}	0^{b}
R. phaseoli	2.49	44.8^{a}	18.0^{a}
M. hapla + R. phaseoli	2.38	50.7^{a}	21.3^{a}

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]Average number of seven replicates.

and nodules per gram root fresh weight was 28% and 37% respectively. Again, the treatment differences were not significant.

In a third experiment, nodulation was again seen to increase in bean plants treated with rhizobia ten days after infestation with M. hapla (Table 34). The average increase in nodules per plant was 13% compared to an 18% increase in number of nodules per gram root fresh weight. Neither of the above treatment differences, analyzed by analysis of variance and Scheffé's test, were significant (Appendix Tables XLVIII, XLIX).

b. Excised Root Experiments

The effect of M. hapla on nodulation of excised bean roots was also studied.

(i) Methods and Materials

Methods used in the preparation of Pencil Pod bean seeds for germination and root excision were the same as described in Part I, Section 3, experiment 3. The type of culture vessel and media used to grow the excised roots were the same as described in Part I, Section 3, experiment 4.

Approximately 250 juveniles of M. hapla were pipetted in 1 ml aliquots along the root system of appropriate treatments. The nematode inoculum was prepared by collecting egg-laying females from dissected roots of tomato plants and transferring these to nutrient solutions in Petri dishes. The females laid eggs continuously in these solutions.

The composition of the medium, described by Ishibashi et al., (1963), was as follows: NaCl 8.0 g/l; KCl 100 mg/l; $CaCl_2$ 100 mg/l; $NaHCO_3$ 200 mg/l; and distilled water 1,000 ml. Females were transferred every two days to fresh solutions. Eggs were collected and allowed to develop until the vermiform shape within the egg was recognizable. At this time the eggs were refrigerated until ready for surface sterilization. Eggs were surface sterilized as described in Part I, Section I, using 0.05% NaOCl (commercial bleach, Javex).

One ml aliquots of freshly prepared R. phaseoli inoculum were distributed to appropriate excised roots.

Inoculum treatments were applied thirteen days after the growth of freshly excised roots was initiated. The treatments,

each replicated six times, were as follows:

1. Excised roots
2. Excised roots + M. hapla
3. Excised roots + R. phaseoli
4. Excised roots + M. hapla + R. phaseoli

All treatments were randomized in a dark, ventilated incubator at 24°C. The experiment was terminated 27 days after addition of rhizobia and nematode inoculum. The root system in each culture vessel was carefully removed and the number of nodules and extent of galling determined.

In a second experiment, the effect of a larger nematode inoculum on nodulation of excised bean roots (cultivar, Slendergreen) was determined. The culture vessel used to grow the excised roots was the same as described in Part I, Section 3, experiment 4. The treatments, each replicated nine times, were the same as in the above experiment. Approximately 2,000 juveniles, obtained from surface sterilized egg masses, were added to the appropriate treatment in one ml volumes. The experiment was terminated after 19 days.

In a third experiment, the effect of a massive number of M. hapla on the nodulation of excised bean roots (cultivar, Pencil Pod) was studied. Ten ml aliquots each containing a total of approximately 5,000 juveniles were pipetted around excised roots of the correct treatment. Rhizobium inoculum was added at the rate of 5 ml per excised root system.

Treatments corresponded to those in the above two experiments. The experiment was terminated after 21 days.

(ii) Results

The data of the above experiments are presented in Tables 35, 36 and 37.

Inoculation of excised bean roots with R. phaseoli and low numbers of M. hapla resulted in an increase (not significant, Appendix Table L) in the number of nodules compared to excised roots inoculated with Rhizobium alone (Table 35). This increase in formation of nodules amounted to an average of 54% per plant. The majority of nodules formed in either of the above treatments appeared immature. No attempt was made to determine fresh weights of each root system. The amount of root galling observed was slight. In roots treated with M. hapla, females had completed the final moult and were almost fully mature. However in the combined inoculum treatment, development had advanced to the egg laying stage. In order to observe whether sufficient nutrients were being obtained by the females to support viable egg production, several eggs were collected and incubated in a small amount of water in Petri dishes. After 12 days motile juveniles were observed, indicating that M. hapla was capable of reproducing on excised bean roots.

The effect of a somewhat larger nematode inoculum (2,000 juveniles per root system) on root growth and nodulation is shown in Table 36. The average number of nodules forming on

Table 35. The effect of low inoculum[2] numbers of M. hapla on nodulation of excised bean roots

Treatment	Av. No. Nodules[3]
Control	0[b1]
M. hapla	0[b]
R. phaseoli	31.8[a]
M. hapla + R. phaseoli	49.0[a]

[1]Any two means in a column with the same superscript are not significantly different (P≤0.05).

[2]250 juveniles per excised root system.

[3]Average of six replicates.

excised roots inoculated with M. hapla and R. phaseoli increased 24% per root system and 15% per gram root fresh weight, compared with roots treated with R. phaseoli alone. Neither of these treatment effects were significantly different, however (Appendix Tables LI, LII). Relatively few galls were noted on the plant roots. The galls were small and female development had proceeded to the completion of moulting.

In testing the effect of 5,000 juveniles of M. hapla per root system on the formation of nodules by R. phaseoli, larger numbers of nodules were observed in the nematode treated roots than in those treated with Rhizobium alone (Table 37). The average increase in nodules per root and nodules per gram root

Table 36. The effect of 2,000 juveniles of M. hapla on the nodulation and growth of excised bean roots

Treatment	Av. Root Fresh Wt. (g)[2]	Av. No. Nodules[2]	Av. No. Nodules /g Root Fr. Wt.[2]
Control	0.356	0[b1]	0[b1]
M. hapla	0.394	0[b]	0[b]
R. phaseoli	0.383	29.7[a]	77.5[a]
M. hapla + R. phaseoli	0.412	36.8[a]	89.5[a]

[1] Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2] Average of nine replicates.

fresh weight was 79% and 76%, respectively. These treatment differences were not statistically significant (Appendix Tables LIII, LIV). Gall formation was more extensive than in the previous two experiments. Mature, egg producing females were observed in several galls.

It was noted in each of the above experiments, that when Rhizobium inoculated root systems were removed from the vessels in which they were growing, great difficulty was experienced in separating the silica sand particles from the root mass due to the cohesion and stickiness provided by the bacterial cells. This was not experienced with roots of the control or M. hapla treatments.

Table 37. Effect of 5,000 M. hapla juveniles on nodulation and growth of excised bean roots

Treatment	Av. Root Fresh Wt. (g)[2]	Av. No. Nodules[2]	Av. No. Nodules/g Root Fr. Wt.[2]
Control	0.371	0[b][1]	0[b][1]
M. hapla	0.389	0[b]	0[b]
R. phaseoli	0.379	7.3[a]	19.2[a]
M. hapla + R. phaseoli	0.388	13.1[a]	33.8[a]

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]Average of ten replicates.

5. Systox and Dimethyl Sulfoxide (DMSO) Effects on Infectivity and Development of M. hapla on Beans

Control of plant parasitic nematodes, including species of Meloidogyne, by systemic organophosphate chemicals, has been widely studied. Sasser et al., (1951) and Sasser (1952) showed that soil drenches of Systox resulted in reduction of root-knot of potatoes and tomatoes, with concentrations as low as 0.005%. Elimination of M. hapla from tubers and roots of potatoes was achieved using seed pieces dipped in 800 ppm solutions of Dimethoate (Helton, 1964). Helton (1965) also showed that soils drenched with Dimethoate at 50 to 1800 ppm were effective in reducing root-knot of tomatoes. Phytotoxicity was not observed below 1800 ppm. Effective control of root

parasitic nematodes has been achieved by some workers using foliage applied systemic insecticides, but Sasser et al., (1951) found that foliar sprays of tomato and tobacco seedlings with concentrations of Systox up to 1.0% did not control root-knot and were phytotoxic. Helton (1964) however, was able to show reduction in numbers of root-knot nematodes in roots of potatoes using foliar sprays of Dimethoate at one, two, or four weeks after plant emergence. Later, Helton (1965) reported suppression of root-knot nematodes with foliar sprays of Dimethoate at 1,800 ppm.

Investigations into the use of dimethyl sulfoxide (DMSO) in plant disease control have been concerned with bacterial leaf spot of peaches (Keil et al., 1965, 1967) and peach tree viruses (Pine, 1967). The use of DMSO in research concerning control of plant root pathogens, such as nematodes, fungi, or bacteria, is still a relatively unexplored area for research.

In view of the above findings, the specific objectives of the experiments in this section of the present research were: (1) to investigate the germination of bean seeds in different concentrations of DMSO, (2) to test the effects of bean seed treatment with mixtures of DMSO and Systox on plant growth, (3) to explore the nematicidal effect of foliar applied Systox and DMSO against M. hapla, and (4) to determine the influence of soil drenched with Systox and DMSO on bean root galling and development of M. hapla. Only the experimental data from

studies with M. hapla were subjected to analysis of variance.

a. Effect of DMSO on Germination of Bean Seeds

(i) Methods and Materials

Four lots of 100 Slendergreen bean seeds were surface sterilized in 0.1% $HgCl_2$ for 5 minutes and rinsed five times in sterile distilled water. Taking into account the recommendations of Erdman and Hsieh (1969), that concentrations of less than 0.1% DMSO should be used in research with higher plants, the above seeds were exposed to 100 ml of 0, 0.01, 0.05, or 0.1% DMSO for 5 hours. Afterwards they were rinsed three times in sterile distilled water and plated, five seeds per plate on modified CRMA. The seeds were incubated at room temperature in the laboratory. On the third day, measurements of seed germination were made, and ten seedlings from each treatment were planted in steamed soil in 10 cm clay pots for long term observation of possible phytotoxic effects.

(ii) Results

Bean seed germination was slightly inhibited by all concentrations of DMSO (Table 38), although suppression below the control value amounted to less than 5%.

Table 38. Germination of bean seeds subjected to varying concentrations of DMSO

	Percent DMSO			
	0	0.01	0.05	0.1
% seed germination	79	76	78	75

Plant growth was observed for a period of 25 days. No symptoms of DMSO phytotoxicity were detected in any plants to which DMSO was applied. Foliage colour and plant height were uniform in all treatments.

b. Effect of DMSO and Systox on Plant Growth

(i) Methods and Materials

Twenty Slendergreen bean seeds were placed in 250 ml Erlenmeyer flasks and exposed for 5 hours to solutions of DMSO, Systox, or DMSO-Systox combinations. Concentrations of DMSO and Systox used were 0.01, 0.05, and 0.1%. Mixtures of DMSO and Systox were prepared by combining 25 ml of the following concentrations: 0.01% DMSO with 0.01% Systox; 0.05% DMSO with 0.05% Systox; and 0.1% DMSO with 0.1% Systox. A distilled water control was included. After soaking, the seeds were rinsed in distilled water, surface sterilized, plated, and incubated as in the above experiment. Determination of seed germination was made after three days. Height measurements of five plants selected from Systox and DMSO-Systox treatments planted in clay pots as above, were recorded after four weeks.

(ii) Results

Seed germination was reduced by all concentrations and combinations of DMSO and Systox (Table 39). There was no evidence of synergism between DMSO and Systox. The lower percentages recorded for germination of DMSO treated seeds, compared with the previous experiment, may be attributed to a smaller number of seeds tested. Retardation of plant height was

observed in all Systox and DMSO-Systox treatments. However the amount of growth reduction in beans treated with solutions of DMSO and Systox was less than for Systox alone.

Table 39. Germination of bean seeds exposed to different concentrations of DMSO, Systox, and DMSO-Systox combinations

Treatment	Percentage Seed Germination
Control	70
DMSO, 0.01%	50
DMSO, 0.05%	45
DMSO, 0.1%	55
Systox, 0.01%	35
Systox, 0.05%	35
Systox, 0.1%	60
DMSO + Systox, 0.01%	50
DMSO + Systox, 0.05%	25
DMSO + Systox, 0.1%	50

c. Effect of Foliar Applications of Systox and DMSO on Infectivity of M. hapla on Beans

(i) Methods and Materials

Twenty, 8 cm clay pots were filled with a steamed greenhouse sandy loam soil, depressions were made, and 5,000 juveniles of M. hapla were pipetted in 5 ml amounts, into each depression.

The nematodes were obtained from surface sterilized egg masses. Ten day old Slendergreen bean seedlings, grown in 100 ml beakers of steamed greenhouse soil, were then transplanted to the above nematode infested pots.

Immediately upon seedling transfer, the foliage portion of five plants per treatment were dipped for approximately 5 seconds into 125 ml beakers containing solutions of 0.1% DMSO, 0.1% Systox, 0.1% DMSO + Systox, or distilled water.

The pots were then randomized in a growth cabinet and maintained at 24°C during the light period and 17°C during the dark period, with a cycle of 14 hours light (13,000 lx) and 10 hours darkness.

Plants were harvested after 34 days. Fresh weights of the root systems were determined and the number of nematode galls were counted.

(ii) Results

The effect of foliar treatment of beans with DMSO and Systox, on the amount of root galling by M. hapla, is summarized in Table 40 and Figure 20.

Gall formation was reduced by all chemical treatments. Analysis of variance indicated that treatment differences were not significant, however (Appendix Tables LV, LVI). There were 13%, 28%, and 34% fewer galls per gram root fresh weight in plants treated with DMSO, Systox, and a mixture of DMSO and

Systox, respectively, than the distilled water control. Root galling on a per plant basis was 12%, 43% and 45% lower in plants treated with DMSO, Systox and the combined DMSO-Systox mixture. In this experiment the number of galls per gram root fresh weight in plants treated with DMSO plus Systox was less than in Systox applications. The data, however, does not suggest a synergistic interaction between the two chemicals.

Table 40. Influence of bean foliage treatment with DMSO, Systox, or DMSO-Systox solutions on root weight and galling by M. hapla

Treatment	Fresh Weight (g)[2] Roots	Root Galling[2] Number/Plant	Number/g Root Fr.Wt.
Control[3]	2.4	323[a1]	135[a1]
DMSO	2.4	285[a]	118[a]
Systox	1.9	184[a]	97[a]
DMSO + Systox	2.0	178[a]	89[a]

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]Average of five replicates.

[3]5,000 nematodes/pot as inoculum.

d. Effect of Soil Drench Application of DMSO and Systox on Infectivity and Development of M. hapla on Beans

(i) Methods and Materials

Greenhouse steamed, sandy loam soil was placed into one hundred 10 cm clay pots and compacted slightly by tamping the

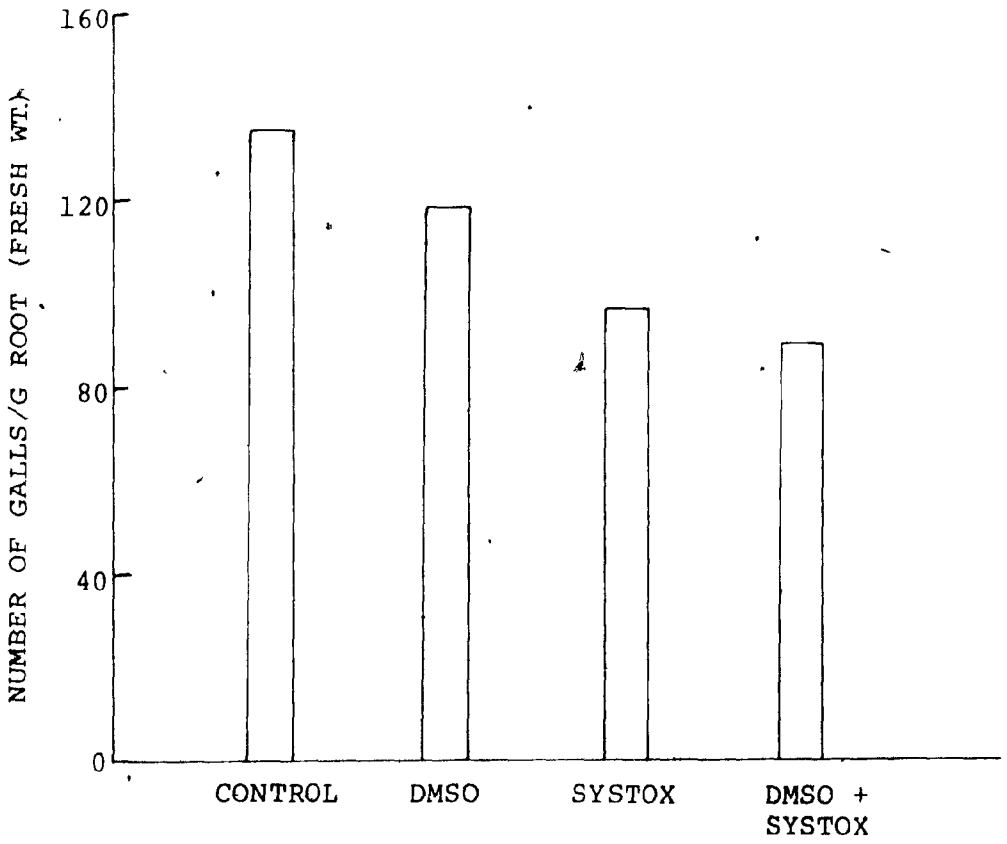


Figure 20. Root galling of beans by M. hapla after foliage treatment with DMSO, Systox or DMSO-Systox solutions.

pots lightly on a bench. Ten ml of a 0.01, 0.05 or 0.1% DMSO, Systox or DMSO-Systox mixture was pipetted to the surface of appropriate pots, and drenched in with 90 ml distilled water. The pots were left overnight for moisture equilibration throughout the soil.

The following day soil impressions were made and 4,000 juveniles of M. hapla pipetted to appropriate treatments. Freshly germinated Slendergreen bean seeds were then sowed in each pot, one per pot.

The treatments, each replicated five times, were as follows:

1. Beans
2. Beans + 0.01% DMSO
3. Beans + 0.05% DMSO
4. Beans + 0.1% DMSO
5. Beans + 0.01% Systox
6. Beans + 0.05% Systox
7. Beans + 0.1% Systox
8. Beans + 0.01% DMSO - Systox
9. Beans + 0.05% DMSO - Systox
10. Beans + 0.1% DMSO - Systox
11. Beans + M. hapla
12. Beans + M. hapla + 0.01% DMSO
13. Beans + M. hapla + 0.05% DMSO
14. Beans + M. hapla + 0.1% DMSO

15. Beans + *M. hapla* + 0.01% Systox
16. Beans + *M. hapla* + 0.05% Systox
17. Beans + *M. hapla* + 0.1% Systox
18. Beans + *M. hapla* + 0.01% DMSO - Systox
19. Beans + *M. hapla* + 0.05% DMSO - Systox
20. Beans + *M. hapla* + 0.1% DMSO - Systox

All treatments were randomized in a greenhouse and watered periodically with distilled water. The average soil temperature during the day was 23°C and 18°C during the night.

After 35 days the experiment was terminated and fresh weight data of foliage and roots were obtained. The roots were fixed in FAA for 48 hr and stained in 0.05% cotton blue lactophenol for 2 min. The number of galls were counted, and using teasing needles, all nematodes were dissected carefully from each root system. The development of *M. hapla* was determined, according to the method of Christie (1946).

(ii) Results

Tables 41 and 42 and Figures 21, 22, 23 and 24 show fresh weights, root infection ratings, and nematode development recorded in the above experiment.

Although certain chemical treatments affected gall formation and development of the nematode, the foliage and root fresh weight data does not reveal an influence of any DMSO, Systox or DMSO-Systox treatment on plant growth (Table 41). Nematode

treated plants usually weighed more (either foliage or root fresh weight) than comparable control plants. At the time of harvest all plants were flowering and in several instances, bean pods of 1.5 cm had formed. The foliage was dark green and there were no symptoms of phytotoxicity.

The number of M. hapla, per plant and per gram root fresh weight, forming galls on bean roots was greatly, and significantly reduced (Appendix Tables LVII, LVIII), by 0.05% and 0.1% concentrations of Systox and by the 0.1% combined DMSO-Systox treatment (Table 41, Figure 21). Although differences between all other treatment means were not significant, root galling was less extensive in the majority of plants receiving chemical treatments compared with the M. hapla controls. Root galling (per gram of root fresh weight) was slightly stimulated over the control (13%) by the 0.01% Systox treatment. The number of nematodes counted in roots treated with various concentrations of combined DMSO-Systox was slightly greater than the number determined in roots receiving Systox solutions alone. Therefore, the data indicate that DMSO does not act as a synergist when combined with Systox, in reducing the infectivity of M. hapla on beans in a soil medium.

After 35 days, very few nematodes dissected from any of the treated roots were at the A stage of development (Table 42). Because of the small numbers, treatment effects could not be detected and analysis of variance for this phase of M. hapla development was not employed.

Table 41. Effect of soil application of DMSO, Systox and DMSO-Systox mixtures on bean root galling by M. hapla

Treatment	Fresh Weight (g)[a] Foliage	Roots	Root Galling[a] No./Plant	No./g Root Fr. Wt.
1. Control	7.1	1.6	0[d1]	0[c1]
2. 0.01% DMSO	9.1	2.7	0[d]	0[c]
3. 0.05% DMSO	8.1	2.0	0[d]	0[c]
4. 0.1% DMSO	6.1	1.3	0[d]	0[c]
5. 0.01% Systox	8.9	2.0	0[d]	0[c]
6. 0.05% Systox	8.6	1.5	0[d]	0[c]
7. 0.1% Systox	7.0	1.6	0[d]	0[c]
8. 0.01% DMSO - Systox	4.6	1.4	0[d]	0[c]
9. 0.05% DMSO - Systox	8.0	1.5	0[d]	0[c]
10. 0.1% DMSO - Systox	7.6	1.8	0[d]	0

(Cont'd)

Table 41. Cont'd.

Treatment	Fresh Weight (g)[a] Foliage	Roots	Root Galling[a] No./Plant	No./g Root Fr. Wt.
11. M. hapla	8.7	2.7	173.0[a][1]	64.1[a][1]
12. M. hapla + 0.01% DMSO	8.8	2.3	130.6[ab]	56.8[a]
13. M. hapla + 0.05% DMSO	6.1	2.4	119.6[abc]	49.7[ab]
14. M. hapla + 0.1% DMSO	8.0	2.6	147.2[ab]	56.4[ab]
15. M. hapla + 0.01% Systox	6.3	1.6	115.8[abc]	72.3[a]
16. M. hapla + 0.05% Systox	10.0	2.1	15.4[cd]	7.3[c]
17. M. hapla + 0.01% Systox	7.2	1.5	5.6[d]	3.7[c]
18. M. hapla + 0.01% DMSO - Systox	7.8	2.6	158.8[ab]	61.0[a]
19. M. hapla + 0.05% DMSO - Systox	7.3	2.1	49.0[abcd]	23.4[abc]
20. M. hapla + 0.1% DMSO -	8.9	2.2	31.0[bcd]	14.1[bc]

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]Average of five replicates.

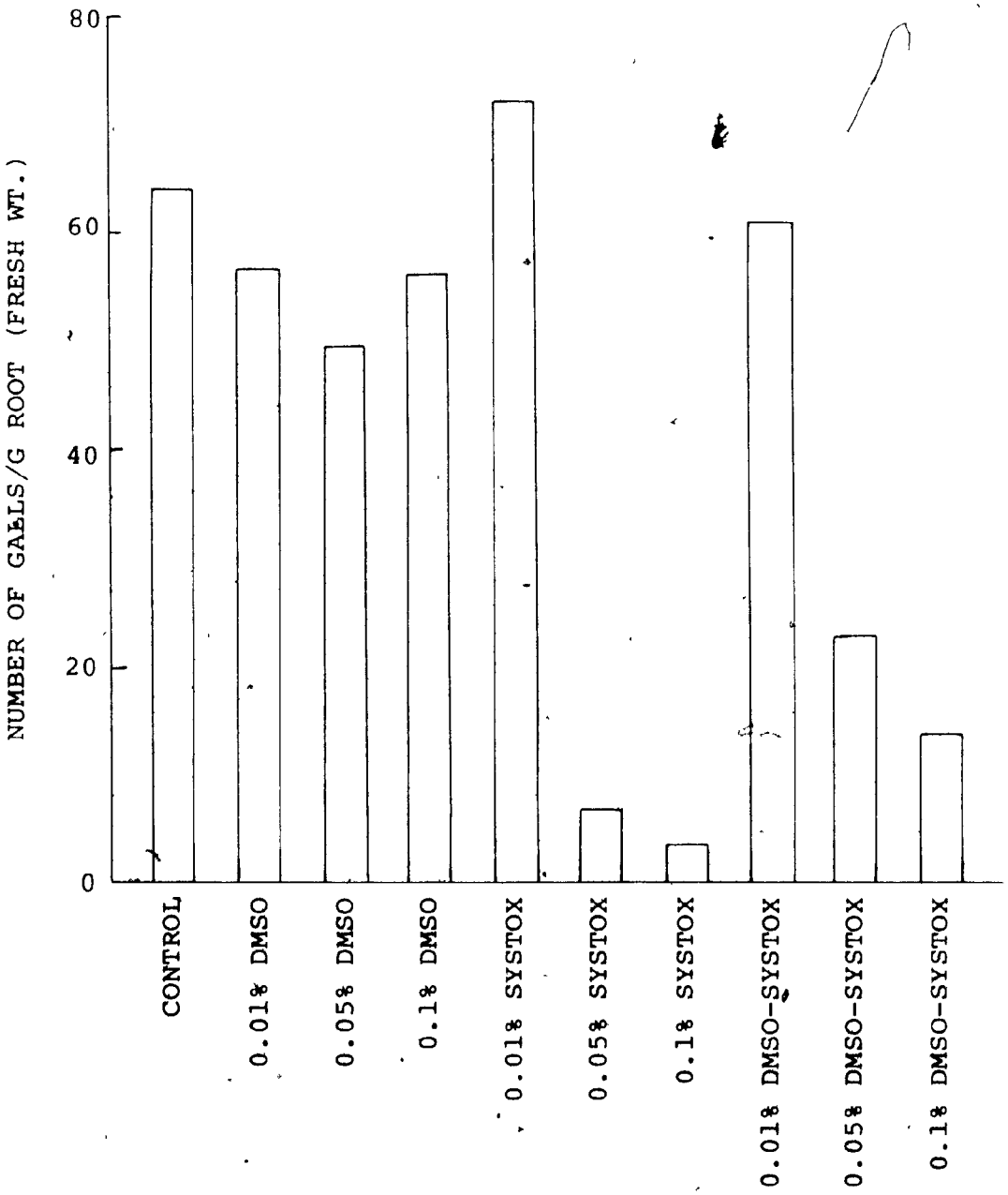


Figure 21. Galling of bean plants in DMSO, Systox and DMXO-Systox treated soil.

Numbers of M. hapla at the B stage (characterized by spike-terminated, hemispherical ends, Figure 11) of morphogensis varied between treatments; the differences, however, were not great enough to be considered significant (Appendix Table LIX). Concentrations of 0.01% and 0.05% DMSO did not influence the number of B stage M. hapla whereas 28% more nematodes were counted in roots treated with 0.1% DMSO, compared to the M. hapla control. Nematodes isolated from roots receiving 0.01% Systox, compared with the control, were 42% greater in number, whereas 0.05% and 0.1% Systox treatments resulted in 75% and 90% fewer M. hapla than in control roots. A pattern similar to that of Systox was observed in plants treated with DMSO-Systox solutions. Eighteen percent more B stage nematodes were isolated from roots treated with 0.01% DMSO-Systox, than in M. hapla controls, whereas 38% and 57% fewer nematodes were determined in roots given 0.05% and 0.1% DMSO-Systox treatments.

Considerable, but not significant (Appendix Table LX), differences in populations of C stage M. hapla occurred between the various treatments, compared with roots receiving M. hapla alone (Table 42). In plants receiving 0.01%, 0.05% and 0.1% DMSO, the numbers of nematodes counted were 20%, 15% and 44% greater, respectively, than in M. hapla inoculated control roots. Nematodes isolated from roots receiving the 0.01% Systox treatment, compared with the control, were 62% greater in number, whereas 0.05% and 0.1% Systox concentrations resulted

Table 42. The effect of DMSO and Systox on development of M. hapla on beans

Treatment[4]	Development[2] of M. hapla A No./g[3] Root Fr.Wt.	B No./g[3] Root Fr.Wt.	C No./g[3] Root Fr.Wt.	D No./g[3] Root Fr.Wt.	E No./g[3] Root Fr.Wt.
Control	0.3	13.0^{ab}[1]	12.2^{a}[1]	31.0^{a}[1]	7.6^{a}[1]
0.01% DMSO	0.2	13.2^{ab}	14.7^{a}	23.7^{ab}	5.0^{a}
0.05% DMSO	0	12.8^{ab}	14.0^{a}	19.9^{abc}	3.1^{a}
0.1% DMSO	0.2	16.6^{ab}	17.6^{a}	21.0^{ab}	1.2^{a}
0.01% Systox	0.1	18.5^{a}	19.8^{a}	26.9^{a}	4.1^{a}
0.05% Systox	0	3.2^{ab}	2.8^{a}	1.3^{c}	0^{a}
0.1% Systox	0.3	1.3^{b}	1.5^{a}	0.7^{c}	0^{a}
0.01% DMSO - Systox	0.2	15.4^{a}	16.1^{a}	26.7^{ab}	1.5^{a}
0.05% DMSO - Systox	0.1	8.0^{ab}	8.1^{a}	6.8^{abc}	0.2^{a}
0.1% DMSO - Systox	0.4	5.6^{ab}	4.0^{a}	3.9^{bc}	0.2^{a}

[1]Any two means in a column with the same superscript are not significantly different ($P \leq 0.05$).

[2]See Figure 11 for illustrations of the developmental stages of M. hapla.

[3]Average of five replicates.

[4]Each treatment contains M. hapla.

in 77% and 88% fewer M. hapla than in control roots. With DMSO-Systox combination treatments, 32% more C stage nematodes were isolated from roots receiving 0.01% DMSO-Systox, than in M. hapla controls, while 34% and 67% fewer nematodes were counted in roots given 0.05% and 0.1% DMSO-Systox treatments.

The largest number (31.0/g root fresh weight) of nematodes in untreated plants was at the D stage of development, which, according to Christie (1946), includes females that have become fully grown but not egg-laying (Figure 11). Three treatments, namely 0.05% and 0.1% concentrations of Systox and the 0.1% combination of DMSO and Systox, were responsible for the significantly fewer (Appendix Table LXI) number of M. hapla forming galls on bean roots treated with these chemicals, compared with control roots inoculated with M. hapla alone. The number of nematodes in roots of plants receiving all other treatments was lower (not significant) than in control roots, indicating a repressive effect of these treatments on the D stage of M. hapla development.

The number of nematodes which developed to the E, or egg-laying stage (Figure 11) was considerably lower (not significant, Appendix Table LXII) in roots receiving 0.1% DMSO, Systox and combined DMSO-Systox, compared with roots treated with only M. hapla. The 0.05% and 0.01% concentrations also reduced the number of nematodes reaching this developmental stage, but not as severely as the 0.1% concentration.

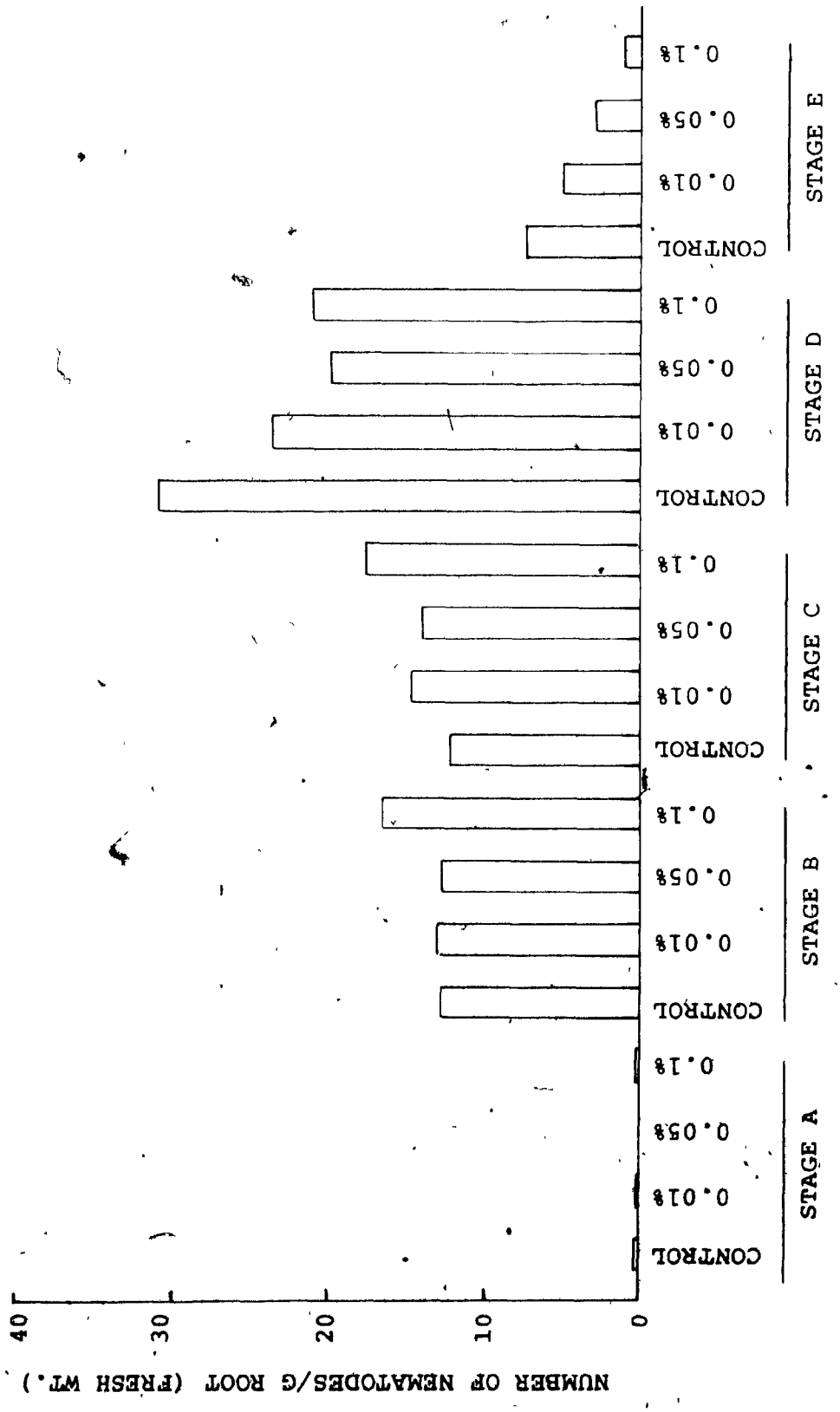


Figure 22. Effect of DMSO on development of M. hapla.

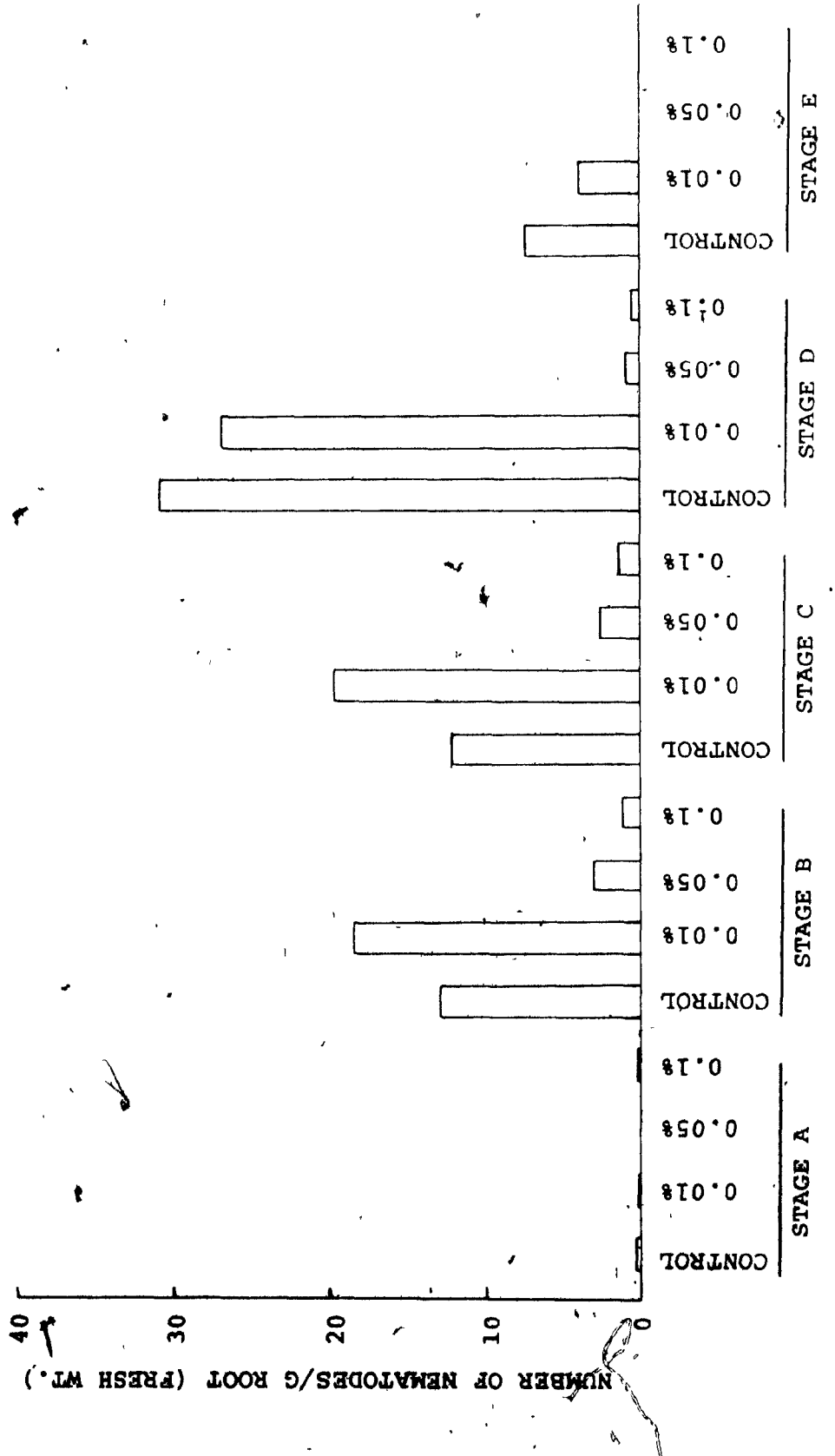


Figure 23. Effect of Systox on development of M. hapla.

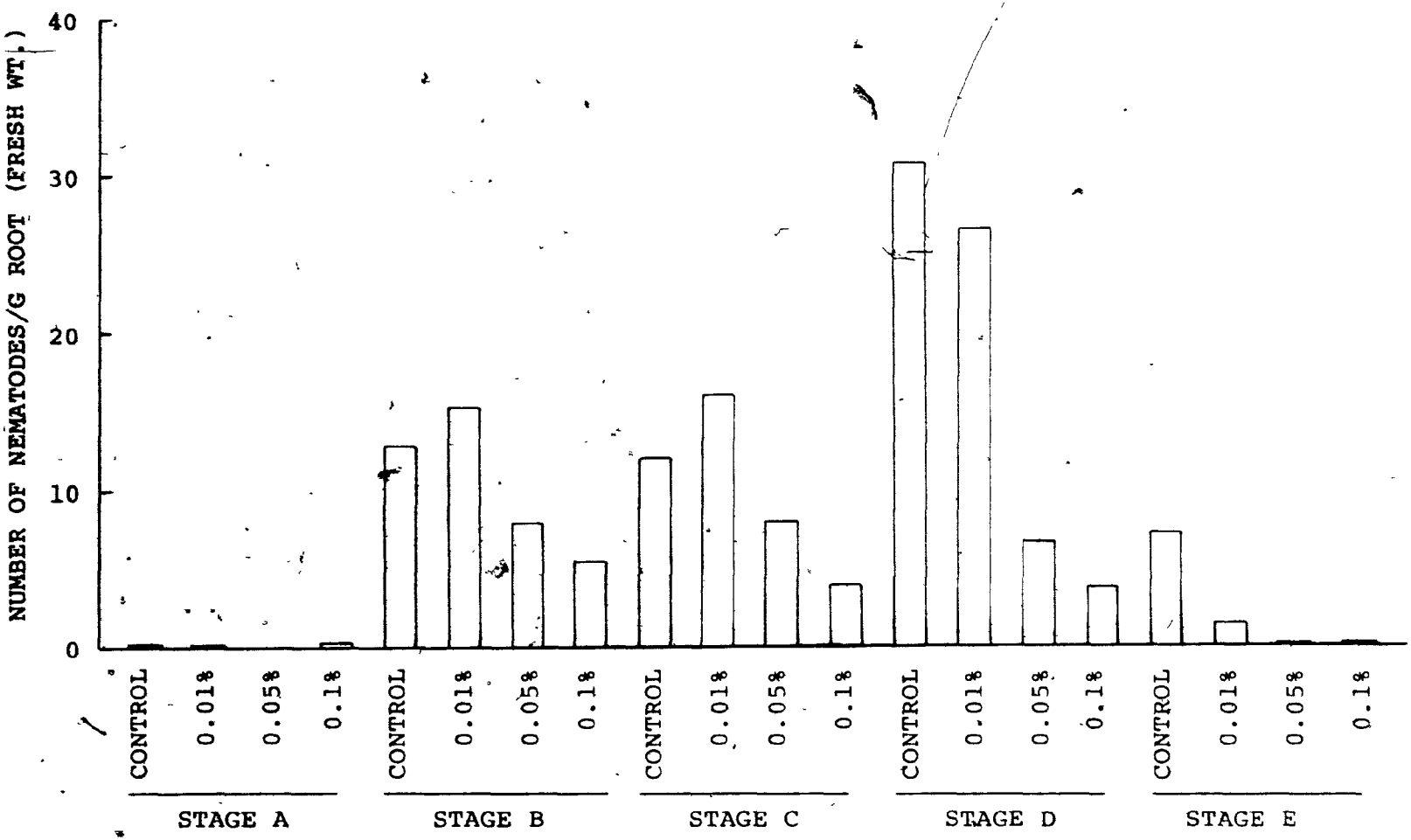


Figure 24. Effect of DMSO-Systox on development of M. hapla.

The number of nematodes which developed to the E, or egg-laying stage (Figure 11) was considerably lower (not significant, Appendix Table LXII) in roots receiving 0.1% DMSO, Systox and combined DMSO-Systox, compared with roots treated with only M. hapla. The 0.05% and 0.01% concentrations also reduced the number of nematodes reaching this developmental stage, but not as severely as the 0.1% concentration.

The influence of various DMSO-Systox combinations on the maturation process of M. hapla was approximately the same as for Systox alone (Fig. 23, 24). The main difference between Systox and DMSO-Systox treatments was that of degree. Systox alone suppressed nematode development to a greater extent than DMSO-Systox combinations. Synergism was not observed in any of the treatments.

DISCUSSION AND CONCLUSIONS FOR PART III

With the exception of the bean cultivars Bountiful and Puregold, the majority of the bean hosts tested were approximately equally suitable for maturation of Meloidogyne hapla. The lowest numbers of mature M. hapla females were isolated from roots of Bountiful and Puregold, indicating some resistance in these plants to M. hapla, compared with other bean hosts. There have been few studies on the development of the root-knot nematode in beans or other legumes, and in only two instances have cultivars used in this work, been tested by other workers.

Christie (1946) found the cultivar Bountiful highly susceptible to the root-knot nematode, which disagrees with these results. At the time of Christie's work, however, the root-knot nematodes were considered to belong to a single species, Heterodera marioni. Thus, the species composition of the inoculum used by Christie is unknown. In the other study wherein similar bean cultivars were tested for resistance to root-knot nematodes, Blazey et al., (1964) observed heavy galling and egg mass production by M. hapla on Cherokee Wax and Contender, reactions which agree with results of this study.

The absence of egg-laying females in roots of all cultivars examined at 21 days was most likely due to temperature. Davide and Triataphyllou (1967a) noted that M. incognita and M. javanica produced eggs on tomato roots as early as 13 days after inoculation at 30 to 35°C, whereas at 15°C egg laying was first detected 60 days after inoculation. Therefore the temperature at which plants were grown in this work, namely 22°C, may not have been high enough to promote the formation and release of eggs by M. hapla within 21 days.

The incorporation of resistance to root-knot nematodes in beans has been achieved by several workers (Blazey et al., 1964; Fassuliotis et al., 1970; Hartmann, 1971; Hutton et al., 1972). However, in view of the results of Blazey et al., (1964), who pointed out that seven bean cultivars most resistant to M. incognita were heavily infested by M. hapla, and Hutton et al., (1972), who discovered resistance in 36 lines of

Phaseolus atropurpureus to several root-knot nematodes except M. hapla, it is evident that resistance in the common bean to this species of root-knot nematode is not well developed. The general susceptibility of most bean cultivars used in this study would support the above conclusion.

Factors other than host cultivar, which have been found to affect root-knot nematode development are temperature (Tyler, 1933; Bird and Wallace, 1965; Davide and Triantaphyllou, 1967a; Griffin, 1969; Fassuliotis et al., 1970), nutrition (Davide and Triantaphyllou, 1967b; Wallace, 1969), inoculum density (Davide and Triantaphyllou, 1967a) and moisture (Couch and Bloom, 1960).

Although a limited number of recent experiments have shown striking evidence of complex interactions involving root-knot nematodes and various saprophytic soil fungi, information concerning interrelationships of parasitic nematodes with saprophytic members of the rhizosphere, such as yeasts, is totally lacking. In this study, Rhodotorula pallida, Candida mycoderma and Torulopsis magnoliae were observed to reduce the number of M. hapla which entered and developed in roots of beans, peas and red clover, compared with plants receiving M. hapla alone. The inability of unicellular organisms such as yeasts to penetrate the roots of higher plants, the favorable development of many M. hapla in yeast treated roots, and the stationary feeding position within roots of the sedentary root-knot group of nematodes, suggest that the lower populations of

M. hapla isolated from yeast-treated roots were due to conditions which retarded penetration of the nematodes. Reduced penetration by M. hapla in the presence of soil yeasts could have been caused by direct interference with nematode metabolism through release of inhibitory substances by yeast inoculum or modification of nematode penetrating sites by plant uptake of yeast metabolites. However, in view of the fact that root-knot nematode juveniles are known to move out of physiologically unsuitable roots (Reynolds et al., 1970), and since yeast cells may have accompanied M. hapla juveniles into the roots, the most tenable reason for the numerical decline of M. hapla in roots of yeast inoculated plants is the migration of this nematode out of the yeast-modified, physiologically unfavorable, root tissues.

The greatest reduction in numbers of M. hapla was noted in R. pallida treatments. Compared with M. hapla treated controls, 54.5% fewer nematodes were dissected from bean roots treated with this yeast, whereas in peas, 39.7% fewer nematodes were isolated. C. mycoderma and T. magnoliae caused decreases of 42.2% and 33.2% respectively, in beans, and 2.8% and 9.7% in peas, compared with M. hapla inoculated controls. Although M. hapla reproduction was not noticeably affected by any of the yeasts tested, the number of nematodes which developed to the Group D stage in beans and to Group E in peas, were very low in roots treated with R. pallida compared to nematode infested roots alone.

Davide and Triantaphyllou (1967b) found that the percentage of males of M. incognita was higher in tomato plants deficient in nitrogen, phosphorous, and potassium compared to plants receiving complete nutrients. They concluded that the nutritional status of the host was related to the development of males in M. incognita. Furthermore, Davide and Triantaphyllou pointed out that it was most likely through changes in the general physiology and nutritional condition of the deficient plants that the change in male percentage occurred. Therefore, in light of the information provided by the above workers, the observed increase in number of males in bean plants receiving yeast inoculum, compared to M. hapla controls, was probably due to deleterious changes in host metabolism caused by the combined activities of yeasts and nematodes within root tissues. The percent of male M. hapla in the bean controls constituted less than 1% of the nematodes isolated, which agrees with the results of Davide and Triantaphyllou (1967a) for M. incognita on tomato.

Galling of bean roots was observed to be consistently greater in plants jointly inoculated with M. hapla and Trichoderma viride than those exposed to the nematode alone. These results can be interpreted on the basis of Powell's (1971b) speculation that extensive physiological changes may occur in roots parasitized by nematodes in the presence of non-pathogenic fungi. If Powell's suggestion is true, then the major function

of T. viride must have involved host root tissue modification, which as a result, provided favorable conditions for the growth and development of M. hapla. Increased stimulation and maintenance of giant cells, which are necessary for normal growth and reproduction of the root-knot nematode (Bird, 1974), could have occurred as a result of interaction between metabolites released by T. viride and the developing syncytia. On the other hand, it is possible that more juveniles entered bean roots infected with a combination of M. hapla and T. viride than in treatments with nematodes alone, since Edmunds and Mai (1966a,b) found greater numbers of Pratylenchus penetrans in roots of alfalfa plants treated with T. viride than in treatments with P. penetrans alone. In view of these results, therefore, it may be concluded that common soil-borne fungi regarded as innocuous on plants in the absence of nematodes, stimulate the amount of root galling by root-knot nematodes, as well as induce extensive necrosis in nematode-altered roots (Powell et al., 1971) and retard plant growth (Edmunds and Mai, 1966b). Furthermore, these results strengthen the hypothesis of Mountain (1965) and Powell (1971a) who suggested that interactions between nematodes and the soil microflora may prove to be more important in the etiology of plant disease than the pathogenicity of plant nematodes per se.

Galling of bean roots by M. hapla was not influenced by a Penicillium sp. but it was notably retarded by Fusarium roseum.

These results indicate that different fungi affect the pathogenicity of M. hapla on beans in different ways. Decreased galling observed on plants inoculated with a combination of M. hapla and F. roseum could have been due to inhibition of root penetration by juveniles of M. hapla, or unfavorable conditions in the root tissues caused by secreted metabolites of the fungus.

The increased galling of bean and pea roots observed in M. hapla and Sclerotinia sclerotiorum treated plants, compared with M. hapla inoculated controls, indicates that this fungus, although capable of plant injury by itself, can increase the amount of root deformation caused by M. hapla. Although it is well known that higher populations of certain nematodes occur in fungus-infected roots (Mountain, 1965), to this writer's knowledge there is only one report (Tu and Cheng, 1971) showing greater root-knot nematode penetration in roots inoculated with a fungal pathogen.

The results of studies on hatching with egg masses of M. hapla show that egg hatching can be greatly affected by different soil-borne fungi. Hatching counts taken 4 days after the initiation of incubation indicate that Penicillium sp., T. viride and F. roseum, increased egg hatch by 26%, 38% and 68%, respectively, whereas S. bataticola suppressed egg hatch by 25%. Stimulation of egg hatch was probably due to fungus secretion

of chemical substances similar to hatching factors (Shepherd and Clarke, 1971) produced by some host plant roots. In addition, enhanced egg hatch may have been due to CO_2 produced by the fungal inoculum on which the egg masses were placed. The concentration of CO_2 has been found critical in hatching of some animal parasitic nematodes (Shepherd and Clarke, 1971). Evidence for a fungal produced hatching factor was given by Giebel (1963), who noted that egg hatch of Heterodera rostochiensis rose from 0.5% to 25% when sterile soil was inoculated with several fungi including Fusarium sp., Trichoderma sp., Penicillium sp. and Zygorhynchus sp. In view of the results of James (1966, 1968), who showed that the fungus causing brown root rot of tomatoes produced substances that inhibited the hatching of H. rostochiensis, the reduced emergence of juveniles from egg masses treated with S. bataticola was probably due to liberation of inhibitory compounds by this fungus. These results suggest, therefore, that hatching may be influenced by non-pathogenic rhizosphere fungi.

The majority of studies regarding interrelationships among nitrogen-fixing bacteria (Rhizobium spp.), plant parasitic nematodes, and their leguminous hosts indicate that suppression of nodule formation is the rule, rather than the exception. In this study, however, increased nodulation was observed repeatedly on roots of whole plants or excised roots receiving a mixture of M. hapla and R. phaseoli, compared with roots

inoculated with R. phaseoli alone. From a thorough search of the literature pertaining directly to studies of inhibition of nodulation by Meloidogyne spp. or Heterodera spp., it is evident that surface disinfestation of egg masses or cysts was not carried out. Since, in the present work, egg masses were regularly treated by immersion for several minutes in Javex containing 5.25% sodium hypochlorite (NaOCl), it is possible that such treatments metabolically altered the maturing juveniles contained within eggs embedded in the egg mass. Although there is not as yet evidence to support the above proposal, sodium hypochlorite has been found to dissolve several nematode structures including cyst walls, eggshells and cuticles, and in low concentrations will cause egg hatch (Shepherd and Clarke, 1971). In addition, certain chemical agents have been discovered to stimulate glandular secretions of juveniles within the egg (Shepherd and Clarke, 1971). In light of these findings, it is proposed that physiologically altered M. hapla juveniles, emerging from sodium hypochlorite treated egg masses, caused increased bean nodulation due to nematode secretion of stimulatory substances. These substances may have been similar to chemicals found stimulatory to root nodulation by Molina and Alexander (1967). Although Taha and Raski (1969) reported reduced numbers of nodules on M. javanica-infected plants, their data (Table 3) also suggests that, under certain conditions, nodulation can be stimulated by root-knot nematodes. These workers found that 30 day old white clover plants receiving

R. trifolii 15 days after inoculation with M. javanica, developed an average of 1,320 nodules, whereas control plants receiving R. trifolii alone, formed only 829 nodules. In addition, Nutman, (1965) pointed out that certain rhizosphere fungi could stimulate root hair infection and nodule formation by Rhizobium.

Although successful culture of Meloidogyne sp. on excised roots of various plants has been accomplished (Zuckerman, 1971), this is the first report showing the reproduction of M. hapla on nodulating excised roots of beans. The greater rate of reproduction of M. hapla on excised bean roots treated with R. phaseoli, than on roots inoculated with the nematode alone, indicates that substances required for nematode maturation and egg formation, such as nitrogen, were supplied more abundantly by the nodulating roots than those parasitized by the nematodes. These results concerning the stimulation of root-knot nematode reproduction by legume bacterial inocula support the findings of Shands and Crittenden (1957), but not those of Ichinohe (1959) and Nigh (1966).

Dimethyl sulfoxide (DMSO), the systemic organophosphate Systox, and a combination of DMSO and Systox, used as foliage treatments at 0.1% concentration, caused reduction of galling on beans by M. hapla. Knotting decreases in the order of 13%, 28% and 34% were observed in plants whose leaves were dipped in DMSO, Systox, and a DMSO-Systox combination, respectively,

compared with M. hapla control plants. Although no work on nematode control has been done with DMSO, the results with Systox are in agreement with studies on Dimethoate by Helton (1964, 1965), and Vamidothion by Dantas and daMatta (1971), who found that foliage applications of these systemic organophosphates effectively reduced infection by the root-knot nematode. Sasser (1951) was unable to control Heterodera rostochiensis on potato, and Meloidogyne sp. on tomato and tobacco seedlings, using foliar sprays of Systox at concentrations ranging from 0.006% to 1.0%. Bergeson (1955), however, obtained good control of Ditylenchus dipsaci on daffodil using a foliage spray of Systox at concentrations of 0.1%, 0.5% and 1.0%. Suppression of root-knot galling of beans by foliage application of Systox was probably due to translocation of Systox itself or metabolized derivatives of this insecticide into the root system. While movement of Systox from roots and stems into leaves of bean seedlings occurs rapidly (Metcalfe et al., 1954), absorption and translocation of this compound from foliage into the root zone is also highly possible in view of the work of Helton (1964), who showed that a related organophosphorous compound, Dimethoate, was translocated systemically from foliage into the tubers of potatoes where it effectively reduced galling due to M. hapla. Studies of the quantitative metabolism of Systox in bean plants by Metcalf et al., (1954), showing that nearly 100 per cent of the thiol- and thiono-isomers of Systox were in metabolized forms within 24 hr of application, indicate

that decreased root galling of beans was due primarily to metabolized forms of both isomers of Systox. The smaller number of galls observed on plants treated with DMSO could have been caused by inhibition of M. hapla penetration and development by DMSO translocated into the root zone, or as suggested by O'Brien (1967), DMSO-solute complexes formed in the plant. Since reduction in root-knot nematode galling by the combined DMSO-Systox treatment was only 6% greater than for Systox alone, it may be concluded that DMSO does not have synergistic properties when used as a solvent for Systox and applied to the leaves of beans.

As a soil drench Systox was more effective than a combination of DMSO and Systox, or DMSO alone, in reduction of galling and reproduction of M. hapla on beans. In all treatments, 0.05% and 0.1% concentrations of chemicals used were more effective than the 0.01% concentration. Galling of plants which grew in soil at 0.05% and 0.1% Systox concentrations, was observed to be reduced by 88.7% and 94.3%, respectively, compared with untreated M. hapla control plants. These results compare favorably with those reported by Sasser (1952), for root-knot reduction on tomato, and Bergeson (1955), for control of Ditylenchus dipsaci on daffodils. One hundred per cent inhibition of reproduction by M. hapla was also obtained with 0.05% and 0.1% Systox concentrations. Although Sasser (1952) was unable to determine the exact nature of Meloidogyne

incognita acrita control using various concentrations of soil-applied Systox, he concluded that control may have been due to inhibition of juvenile hatch and paralysis of hatched juveniles in soil. Since juveniles of M. hapla were used as inoculum in this study, it is most likely that nematode paralysis as observed by Sasser (1952), was responsible for the severe reduction in galling and egg production on beans. The efficacy of soil-applied Systox may also have been due to long term systemic activity of this compound within the roots of beans. Evidence for this latter suggestion was provided by Metcalf et al., (1954) who showed that 60 days after seedling root uptake of Systox, there was an appreciable amount of Systox remaining in the root system despite translocation of much of the compound into stem and leaf tissue. Since there is evidence of a cholinergic nerve system in Meloidogyne (Bird, 1966), it is likely that the effectiveness of Systox was due to inhibition of cholinesterase. Slight reduction in root galling by M. hapla in soils drenched with 0.05% and 0.1% concentrations of DMSO, compared with marked inhibition of reproduction, indicate that this compound is effective in retarding egg development rather than suppressing nematode root penetration. These results would further suggest that DMSO is most effective within the plant tissues either: (1) directly interfering with the later stages of nematode development, (2) forming complexes with plant metabolites which prevent egg formation

by female M. hapla, or (3) stimulating the plant to synthesize substances which are themselves toxic to M. hapla or which combine with DMSO to inhibit egg production. Systox, in combination with DMSO, was less effective in suppressing root galling and nematode development than Systox alone, suggesting that when soil applied, DMSO interferes with Systox activity, possibly by forming less active complexes with soil minerals or within root tissues.

GENERAL DISCUSSION AND CONCLUSIONS

Saprozoic nematode growth, maturation and egg deposition occurred in monoxenic agar culture with several species of Rhizobium. The results suggest that in the natural soil environment, the root nodule bacteria of legumes may play a role in the provision of food for saprozoic nematodes. In view of the abundance of rhizobia around legume host roots (Nutman, 1965), it is unlikely that their populations would be reduced, through nematode feeding, to levels where diminished nodulation would result. Also, results showing development and maturation of saprozoic nematodes on rhizobia consisting of predominantly bacteroid cells suggests that, upon microbial decomposition of nodules in soil, portions of the bacterial contents as well as the smaller number of normal rod-shaped rhizobia, may be consumed by bacterial feeding nematodes. Some of the nitrogen assimilated during nodule life may, therefore, be temporarily immobilized in the cytoplasm of saprozoic nematodes.

Five species of yeasts were shown to support growth and reproduction of an unidentified species of Rhabditis. It appears that a part of the nutritional requirements of free-living soil nematodes such as Rhabditis sp. may be derived from ingestion of yeasts. This should be more extensively studied, especially in relation to the rhizosphere, where numbers and kinds of soil yeasts are greatest (Babeva and Saveleva, 1963; Babeva and Belyanin, 1966).

Demonstration of juvenile survival for 21 days in the absence of bacteria or yeasts indicates that, in nature, this stage of the life cycle of saprozoic nematodes may be capable of withstanding long periods of time without food. This may be a factor ensuring their survival during periods of food stress.

Although it is generally accepted that freeliving soil nematodes are consumers of bacteria, little emphasis has been placed on the disseminatory function of these soil organisms. The movement of rhizobia from areas distant to legume roots, into the rhizosphere, was shown to be facilitated by saprozoic nematodes. As a result, distribution of rhizobia along the growing root system may have become more extensive and the number of nodules increased. Dissemination of members of the soil community by saprozoic nematodes is not confined solely to beneficial organisms such as Rhizobium spp. Studies by Soloveva (1965) and Chantanao and Jensen (1969b) indicate that plant pathogenic fungi and bacteria are carried through soil by freeliving nematodes. Since it is well known that untreated soil around plant roots contains pathogenic microorganisms (Jensen, 1967), the presence of saprozoic nematodes, capable of transporting these pathogens into the rhizosphere and onto the rhizoplane surface, should not be ignored by root-disease investigators using natural soil as a rooting medium.

Egg hatch of a Rhabditis sp. was stimulated in nutrient solutions, compared with distilled water. Increased egg hatch may have been due to excreted microbial metabolites since the solutions became contaminated due to the necessity of opening the dishes for counting purposes. This suggests that certain microorganisms, which adhere, or are adjacent to, freeliving nematode eggs in soil, may affect the hatching process. This may occur directly through release of compounds which stimulate hatching by promoting enzymatic activity within the egg, or indirectly by external enzymatic weakening of the egg cuticle.

Considerable variation observed between selective preference and multiplication of Aphelenchus avenae with different fungi implies that the result of each association is due to characteristics which are peculiar to that particular association. Though mycophagous nematodes in natural soil are influenced by several factors simultaneously, the use of autoclaved soil in experiments with A. avenae may simulate natural soil conditions. Hence, a preferential aggregation of A. avenae around a fungus may be representative of the expected response in natural soil. Furthermore, the suitability of a particular fungus for A. avenae population increase can be expected to play a part in the population dynamics of this nematode in soil.

Suppression of nodule formation on excised bean roots and severe rootlet damage by A. avenae in the presence of R. phaseoli indicate that, under certain conditions, this nematode can detrimentally affect roots of higher plants. The interaction of mycophagous nematodes such as A. avenae, and soil bacteria, normally regarded as innocuous or beneficial to plant growth, merits further study. This would apply particularly to situations where soil is treated with fungicides which reduce or eliminate the normal food source of mycophagous nematodes.

A. avenae eliminated damping-off of beans caused by Sclerotinia sclerotiorum when nematode and fungus inocula were added simultaneously. Since damping-off of seedlings caused by S. sclerotiorum is more severe at lower soil temperatures (Porth, 1966), investigations with several soil temperatures and time intervals between the addition of nematodes and fungus, may provide a better understanding of the role of this nematode in the control of damping-off caused by S. sclerotiorum.

Resistance of Phaseolus vulgaris to root-knot nematodes has been observed by several workers (Blazey et al., 1964; Fassuliotis et al., 1970; Hartmann, 1971; Hutton et al., 1972). The susceptibility of all bean cultivars tested in this study to Meloidogyne hapla, and the discovery by Blazey et al., (1964) and Hutton et al., (1972) that bean resistance to species of root-knot nematodes except M. hapla exists, suggests that plant

breeders should include M. hapla in breeding programmes designed to develop general resistance to root-knot nematodes.

Different yeasts reduced the number of M. hapla which entered and developed in bean roots. On the other hand, the number of galls caused by M. hapla were increased by Trichoderma viride and S. sclerotiorum. Furthermore, egg hatch of M. hapla was influenced by several common soil fungi. Egg hatch was stimulated by a Penicillium sp., T. viride and Fusarium roseum whereas Sclerotium bataticola inhibited egg hatch. These results raise the question as to the interrelationships between root-knot nematodes and the wide variety of microorganisms in the rhizosphere of plants. This aspect of microbial interaction deserves further study because of the ubiquity of these groups of microorganisms and their possible role in root diseases.

M. hapla caused an increase in the number of nodules on beans using whole plants or excised roots. Many reports have indicated inhibition of nodulation by plant parasitic nematodes, but in view of the results of Taha and Raski (1969), who noted increased nodulation under certain conditions of M. javanica, Rhizobium trifolii and white clover interaction, and Lehman et al., (1971), who showed that certain races of Heterodera glycines do not affect nodulation, the relationship between strains of nodule bacteria and root-knot nematodes needs further examination.

Foliage application of a combination of DMSO and Systox at 0.1% was more effective than either compound separately, in reducing galling of beans by M. hapla. On the other hand, Systox applied as a soil drench treatment, was more effective in reducing galling than the combination or DMSO alone. The results indicate that DMSO and related compounds should be tested for systemic nematicidal effects in combination with other organophosphate nematicides applied as foliage sprays. It would also appear, at least from these experiments, that DMSO is of no value as a synergist, when soil applied for control of root-knot nematodes.

SUMMARY

1. Species of the saprozoic nematodes Rhabditis and Chiloplacus grew and produced eggs in monoxenic culture with Rhizobium phaseoli, R. trifolii and R. leguminosarum. Egg deposition began at 12 days and was rapid at 15 days. Nematode growth was approximately equal on all species of Rhizobium. Hatched juveniles on control plates were alive and motile for periods of 21 days. Morphological development of Rhabditis sp. was also observed in bacteroid cultures of Rhizobium spp. grown on Nutrient Agar.

2. Maturation and egg deposition of a Rhabditis sp. occurred on the yeasts Candida mycoderma, Hansenula schneggi, Rhodotorula pallida, Saccharomyces cerevisiae and Torulopsis magnoliae cultured monoxenically on a modified Wickerham's medium. Egg production was slow at 15 days, becoming rapid at 18 days. Nematode development was most rapid on R. pallida whereas S. cerevisiae retarded the development of this nematode.

3. Transport of viable cells of Rhizobium phaseoli into the rhizospheres of intact or excised bean roots, was aided by species of Chiloplacus, Cephalobus and Rhabditis. Large numbers of a Rhabditis sp. did not affect the number of nodules formed on intact bean plants.

4. Egg hatch of surface sterilized eggs of a Rhabditis sp. in sterile distilled water increased with increasing temperature from 15°C to 30°C. Egg hatch was greatest at 30°C and was stimulated by certain nutrients and contaminating microorganisms.

5. Aphelenchus avenae was attracted strongly to colonies of soil-or Water Agar-cultured Verticillium albo-atrum, Fusarium culmorum, Verticillium cinnabarinum and Trichoderma viride whereas Sclerotinia sclerotiorum, Sclerotium bataticola and Helminthosporium sativum were found unattractive by this nematode.

6. Progeny numbers of A. avenae were greatest at 16 days on F. culmorum, S. bataticola, Fusarium sp. and T. viride cultured in a glucose amended soil medium. A Pullularia sp. and two actinomycete isolates did not support reproduction of this nematode. Fungi preferred by A. avenae were not necessarily the most suitable hosts for progeny increase.

7. Nodulation of excised bean roots was suppressed by A. avenae. Nematode activities including rapidness of movement, root-hair and root surface feeding and egg deposition were stimulated by R. phaseoli. Root damage by A. avenae was greatest when cells of R. phaseoli were present.

8. Populations of 3,300 to 13,200 individuals of A. avenae controlled pre-emergence damping-off of beans induced by Sclerotinia sclerotiorum.

9. Eleven cultivars of the common bean (Phaseolus vulgaris) were found to be susceptible to Meloidogyne hapla. There were no significant differences in development of M. hapla between cultivars.

10. Entry and development of M. hapla in roots of beans, peas and clover was suppressed by the yeasts Torulopsis magnoliae, Rhodotorula pallida and Candida mycoderma.

11. The number of galls on bean or pea roots caused by M. hapla was increased by the saprophyte Trichoderma viride and the pathogen Sclerotinia sclerotiorum.

12. M. hapla juvenile hatch from egg masses was stimulated by a Penicillium sp., T. viride and Fusarium roseum whereas egg hatch was suppressed by Sclerotium batatícola.

13. In the presence of Rhizobium phaseoli, M. hapla caused an increase in the number of nodules both on intact bean plant roots and on excised bean roots.

14. Both foliage treatment and soil drenches of DMSO, Systox and a combination of DMSO and Systox ranging from 0.01

to 0.1% suppressed root-knot of beans caused by M. hapla. Soil drench application of the above chemicals inhibited development and reduced reproduction of this nematode. There was no evidence of phytotoxicity at the concentrations used, either for foliar or soil application.

CLAIMS OF ORIGINAL WORK

1. Found evidence that several Rhizobium spp. and yeasts supported growth, development and egg production of saprozoic nematodes in monoxenic culture.

2. Studied the transport of R. phaseoli by saprozoic nematodes to the rhizospheres of intact and excised bean roots.

3. Studied the effect of large numbers of saprozoic nematodes on nodule formation on beans.

4. Studied the effect of temperature on egg hatch of a species of Rhabditis.

5. Found evidence of selective tendencies of Aphelenchus avenae for certain fungi in soil.

6. Studied the effect of fungal hosts cultured in soil on the fecundity of A. avenae.

7. Found evidence of interaction between A. avenae, R. phaseoli and excised bean roots.

8. Found evidence that A. avenae controlled damping-off of beans caused by Sclerotinia sclerotiorum.

9. Studied the development of Meloidogyne hapla in the roots of several cultivars of beans.

10. Found evidence that yeasts suppressed entry and development of *M. hapla* in beans, peas and clover.

11. Found evidence of interaction between *M. hapla*, *Trichoderma viride* and *S: sclerotiorum*.

12. Found evidence that *M. hapla* juvenile hatch from egg masses was influenced by soil fungi.

13. Studied the effect of *M. hapla* on the formation of nodules on intact and excised bean roots.

14. Studied the use of a systemic insecticide and a solvent for the control of *M. hapla* on beans.

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APPENDIX

Tables of Statistical Analysis

Table 1. The effect of Chiloplacus on the movement of R. phaseoli into the rhizosphere of beans

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	1.35
Replication	5	0.56
Residual	15	0.71

Table II. The effect of Cephalobus on the movement of R. phaseoli into the rhizosphere of excised bean roots

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	6.73**
Replication	5	1.17
Residual	15	1.18

**$P \leq 0.01$

Table III. Effect of temperature on egg hatch of a Rhabditis sp.

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	0.85*
Replication	3	0.43
Residual	9	0.18

*$P \leq 0.05$

Table IV. Selection of different soil fungi by A. avenae in a PDA medium

Source of Variation	Degrees of Freedom	Mean Square
Treatment	6	3.54**
Replication	3	0.64
Residual	18	0.46

**$P \leq 0.01$

Table V. Selection at 18 hr of different soil fungi by A. avenae in a WA medium

Source of Variation	Degrees of Freedom	Mean Square
Treatment	12	2.57**
Replication	3	1.61**
Residual	36	0.67

**P≤0.01

Table VI. Selection at 24 hr of different soil fungi by A. avenae in a WA medium

Source of Variation	Degrees of Freedom	Mean Square
Treatment	12	5.46**
Replication	3	1.18
Residual	36	1.46

**P≤0.01

Table VII. Selection of different soil fungi by A. avenae in a glucose amended soil medium

Source of Variation	Degrees of Freedom	Mean Square
Treatment	12	6.96**
Replication	3	0.80
Residual	36	0.84

**P≤0.01

Table VIII. The effect at 4 days of fungi and actinomycetes on the fecundity of A. avenae

Source of Variation	Degrees of Freedom	Mean Square
Treatment	7	86.74**
Replication	2	0.67
Residual	14	4.50

**P≤0.01

Table IX. The effect at 8 days of fungi and actinomycetes on the fecundity of A. avenae

Source of Variation	Degrees of Freedom	Mean Square
Treatment	7	1651.03**
Replication	2	1.20
Residual	14	13.91

**P≤0.01

Table X. The effect at 12 days of fungi and actinomycetes on the fecundity of A. avenae

Source of Variation	Degrees of Freedom	Mean Square
Treatment	7	1448.07**
Replication	2	22.48
Residual	14	18.90

**P≤0.01

Table XI. The effect at 16 days of fungi and actinomycetes on the fecundity of A. avenae

Source of Variation	Degrees of Freedom	Mean Square
Treatment	7	1041.07
Replication	2	19.19
Residual	14	32.46

**P≤0.01

Table XII. The effect at 4 days of different fungi on the fecundity of A. avenae

Source of Variation	Degrees of Freedom	Mean Square
Treatment	6	0.017**
Replication	2	0.001
Residual	12	0.004

**P≤0.01

Table XIII. The effect at 8 days of different fungi on the fecundity of A. avenae

Source of Variation	Degrees of Freedom	Mean Square
Treatment	6	2.65**
Replication	2	0.06
Residual	12	0.11

**P≤0.01

Table XIV. The effect at 12 days of different fungi on the fecundity of A. avenae

Source of Variation	Degrees of Freedom	Mean Square
Treatment	6	202.89**
Replication	2	1.31
Residual	12	0.81

**P≤0.01

Table XV. The effect at 16 days of different fungi on the fecundity of A. avenae

Source of Variation	Degrees of Freedom	Mean Square
Treatment	6	751.15**
Replication	2	4.31
Residual	12	3.01

**P≤0.01

Table XVI. The effect of A. avenae on the number of nodules per root on excised bean roots

Source of Variation	Degrees of Freedom	Mean Square
Treatment	7	10.39**
Replication	4	0.45
Residual	28	1.43

**P≤0.01

Table XVII. The effect of A. avenae on the number of nodules per gram of root on excised bean roots

Source of Variation	Degrees of Freedom	Mean Square
Treatment	7	34.88**
Replication	4	1.84
Residual	28	3.39

**$P \leq 0.01$

Table XVIII. The effect of bean cultivar on the A stage of M. hapla development at 14 days

Source of Variation	Degrees of Freedom	Mean Square
Treatment	10	42.43
Replication	1	137.50
Residual	10	57.20

Table XIX. The effect of bean cultivar on the B stage of M. hapla development at 14 days

Source of Variation	Degrees of Freedom	Mean Square
Treatment	10	11.03
Replication	1	10.64
Residual	10	3.79

Table XX. The effect of bean cultivar on the B stage of M. hapla development at 21 days

Source of Variation	Degrees of Freedom	Mean Square
Treatment	10	3.05
Replication	1	2.49
Residual	10	0.66

Table XXI. The effect of bean cultivar on the C stage of M. hapla development at 21 days

Source of Variation	Degrees of Freedom	Mean Square
Treatment	10	1.16
Replication	1	0.17
Residual	10	0.27

Table XXII. The effect of bean cultivar on the D stage of M. hapla development at 21 days

Source of Variation	Degrees of Freedom	Mean Square
Treatment	10	2.25
Replication	1	1.32
Residual	10	0.72

Table XXIII. The effect of soil yeasts on the total number of M. hapla invading roots of beans

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	14.72
Replication	5	10.80
Residual	15	8.35

Table XXIV. The effect of soil yeasts on the total number of M. hapla invading roots of peas

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	12.71
Replication	5	14.22
Residual	15	4.85

Table XXV. The effect of soil yeasts on the B stage of M. hapla development in beans

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	7.50*
Replication	5	1.52
Residual	15	1.91

*P≤0.05

Table XXVI. The effect of soil yeasts on the B stage of M. hapla development in peas

Source of Variation*	Degrees of Freedom	Mean Square
Treatment	3	17.13**
Replication	5	11.76
Residual	15	2.65

**P≤0.01

Table XXVII. The effect of soil yeasts on the C stage of M. hapla development in beans

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	1.52
Replication	5	3.18
Residual	15	2.39

Table XXVIII. The effect of soil yeasts on the C stage of M. hapla development in peas

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	0.34
Replication	5	5.21
Residual	15	2.11

Table XXIX. The effect of soil yeasts on the D stage of M. hapla development in beans

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	9.23
Replication	5	8.76
Residual	15	7.44

Table XXX. The effect of soil yeasts on the D stage of M. hapla development in peas

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	17.70**
Replication	5	3.31
Residual	15	2.74

**$p \leq 0.01$

Table XXXI. The effect of soil yeasts on the E stage of M. hapla development in beans

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	1.81
Replication	5	1.24
Residual	15	1.87

Table XXXII. The effect of soil yeasts on the E stage of M. hapla development in peas

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	1.63
Replication	5	2.32
Residual	15	8.55

Table XXXIII. The effect of soil fungi on the number of galls per gram of bean root caused by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	7	46.94**
Replication	5	0.14
Residual	35	0.60

**P≤0.01

Table XXXIV. The effect of soil fungi on the number of galls per bean root caused by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	7	138.18**
Replication	5	0.14
Residual	35	1.21

**P≤0.01

Table XXXV. The effect of T. viride on the number of galls per gram of bean root caused by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	129.28**
Replication	5	2.01
Residual	15	0.93

**P≤0.01

Table XXXVI. The effect of T. viride on the number of galls per bean root caused by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	720.17**
Replication	5	11.87
Residual	15	6.73

**P≤0.01

Table XXXVII. The effect of 4,000 M. hapla and T. viride on the number of galls per gram of bean root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	172.16**
Replication	5	1.63
Residual	15	1.78

**P≤0.01

Table XXXVIII. The effect of 4,000 M. hapla and T. viride on the number of galls per bean root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	806.95**
Replication	5	32.44
Residual	15	16.01

**P≤0.01

Table XXXIX. The effect of S. sclerotiorum on the number of galls per bean plant caused by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	601.17**
Replication	6	3.21
Residual	18	5.28

** P≤0.01

Table XL. The effect of S. sclerotiorum on the number of galls per gram of bean root caused by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	137.90**
Replication	6	1.28
Residual	18	0.97

**P≤0.01

Table XLI. The effect of S. sclerotiorum on the number of galls per gram of pea root incited by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	1228.87**
Replication	7	10.37
Residual	21	10.52

**P≤0.01

Table XLII. The effect of S. sclerotiorum on the number of galls per pea root incited by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	1277.85**
Replication	7	62.62
Residual	21	25.87

**P≤0.01

Table XLIII. Influence of different fungi on egg hatch of M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	4	211.59**
Replication	9	35.65
Residual	36	29.40

**P≤0.01

Table XLIV. The effect of M. hapla egg mass number on the number of nodules per bean root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	7	74.34**
Replication	5	1.27
Residual	35	2.31

**P≤0.01

Table XLV. The effect of M. hapla egg mass number on the number of nodules per gram of bean root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	7	32.95**
Replication	5	0.23
Residual	35	0.49

**P≤0.01

Table XLVI. The effect of M. hapla and delayed rhizobial inoculation on number of nodules per bean root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	5	63.86**
Replication	5	2.55
Residual	25	2.87

**P≤0.01

Table XLVII. The effect of M. hapla and delayed rhizobial inoculation on number of nodules per gram of bean root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	5	18.54**
Replication	5	0.52
Residual	25	0.87

**P≤0.01

Table XLVIII. The effect of bean inoculation with R. phaseoli 10 days after exposure to M. hapla on number of nodules per root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	69.63**
Replication	6	0.77
Residual	18	0.83

**P≤0.01

Table XLIX. The effect of bean inoculation with R. phaseoli 10 days after exposure to M. hapla on number of nodules per gram of root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	26.04**
Replication	6	0.17
Residual	18	0.13

**P≤0.01

Table L. The effect of 250 M. hapla on nodulation of excised bean roots

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	56.12**
Replication	5	1.00
Residual	15	1.49

**P≤0.01

Table LI. The effect of 2,000 M. hapla on number of nodules per excised bean root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	67.93**
Replication	8	0.78
Residual	24	0.63

**P≤0.01

Table LII. The effect of 2,000 M. hapla on number of nodules per gram of excised bean root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	197.85**
Replication	8	2.25
Residual	24	1.47

**P≤0.01

Table LIII. The effect of 5,000 M. hapla on number of nodules per excised bean root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	15.76**
Replication	9	0.50
Residual	27	0.81

**$P \leq 0.01$

Table LIV. The effect of 5,000 M. hapla on number of nodules per gram of excised bean root

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	50.86**
Replication	9	1.43
Residual	27	2.14

**$P \leq 0.01$

Table LV. The effect of DMSO, Systox and DMSO-Systox foliage treatment of beans on number of galls per root caused by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	34.43
Replication	4	23.17
Residual	12	25.57

Table LVI. The effect of DMSO, Systox and DMSO-Systox foliage treatment of beans on number of galls per gram of root caused by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	3	8.92
Replication	4	1.29
Residual	12	4.62

Table LVII. The effect of soil applied DMSO, Systox and DMSO-Systox mixtures on number of galls per bean root caused by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	19	112.54**
Replication	4	2.15
Residual	76	4.03

**P≤0.01

Table LVIII. The effect of soil applied DMSO, Systox and DMSO-Systox mixtures on number of galls per gram of bean root caused by M. hapla

Source of Variation	Degrees of Freedom	Mean Square
Treatment	19	46.10**
Replication	4	0.27
Residual	76	1.30

**P≤0.01

Table LIX. The effect of DMSO, Systox and DMSO-Systox on the number of B stage M. hapla in beans

Source of Variation	Degrees of Freedom	Mean Square
Treatment	9	5.08**
Replication	4	0.12
Residual	36	0.94

**P≤0.01

Table LX. The effect of DMSO, Systox and DMSO-Systox on the number of C stage M. hapla in beans

Source of Variation	Degrees of Freedom	Mean Square
Treatment	9	5.66**
Replication	4	0.43
Residual	36	1.27

**P≤0.01

Table LXI. The effect of DMSO; Systox and DMSO-Systox on the number of D stage M. hapla in beans

Source of Variation	Degrees of Freedom	Mean Square
Treatment	9	13.22**
Replication	4	0.68
Residual	36	1.36

**P≤0.01

Table LXII. The effect of DMSO, Systox and DMSO-Systox on the number of E stage M. hapla in beans

Source of Variation	Degrees of Freedom	Mean Square
Treatment	9	1.84**
Replication	4	0.71
Residual	36	0.43

**P≤0.01